

Effects on Cattle from
Exposure to Sewage Sludge

Metropolitan Denver Sewage
Disposal District No. 1, CO

Prepared for

Municipal Environmental Research Lab.
Cincinnati, OH

Feb 83



EPA-600/2-83-012
February 1983

EFFECTS ON CATTLE FROM EXPOSURE TO SEWAGE SLUDGE

by

John C. Baxter
Metropolitan Denver Sewage Disposal District No. 1
Denver, Colorado 80229

Donald Johnson
Eldon Kienholz
Department of Animal Science
Colorado State University
Fort Collins, Colorado 80705

Wylie D. Burge
William N. Cramer
Agricultural Environmental Quality Institute
U.S. Department of Agriculture
Beltsville, Maryland 20705

Contract No. 68-03-2210

Project Officers

Gerald Stern
Wastewater Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

George L. Braude
Office of the Associate Director for Physical Science
Bureau of Foods
U.S. Food and Drug Administration
Washington, D.C. 20204

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA (Please read instructions on the reverse before completing)		
1. REPORT NO. EPA-600/2-83-012	2.	3. RECIPIENT'S ACCESSION NO. PB83 170589
4. TITLE AND SUBTITLE Effects on Cattle from Exposure to Sewage Sludge		5. REPORT DATE February 1983
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) John C. Baxter; D. Johnson, E. Kienholz (Colorado State University) W.D. Burge, W.N. Cramer (U.S.D.A.)		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Metropolitan Denver Sewage Disposal District No. 1 6450 York Street Denver, CO 80229		10. PROGRAM ELEMENT NO. AZB1B, D.U.B-113, Task C/12
		11. CONTRACT/GRANT NO. 68-03-2210
12. SPONSORING AGENCY NAME AND ADDRESS Municipal Environmental Research Laboratory - Cin., OH Office of Research and Development U.S. Environmental Protection Agency Cincinnati, OH 45268		13. TYPE OF REPORT AND PERIOD COVERED Final
		14. SPONSORING AGENCY CODE EPA/600/14
15. SUPPLEMENTARY NOTES Project Officers - Gerald Stern Telephone Number 513-684-7654 George Braude Telephone Number 202-245-1152		
16. ABSTRACT Soils, forages, and cattle grazing on a sludge disposal site were examined for trace metals and persistent organics. Soils at the disposal site had increased concentrations of Zn, Cu, Ni, Cd, and Pb. Forages from sludge applied soils had higher levels of Zn, Cd, Cu, and Ni and lower Pb concentrations than forages from soils that had not received sludge. Cattle grazing on the sludge disposal site were healthy with no signs of pathology. Tissues from these cattle did not show elevated levels of metals or persistent organics when compared with cattle not exposed to sewage sludge. Sewage sludge was added to the diets of cattle to simulate worst case conditions of cattle grazing sludge fertilized pastures. The sludge had no positive or negative effects on cattle health or performance but did act as a diet diluent. The direct ingestion of sewage sludge led to increased levels of Cd and Pb in kidney and liver tissues. The amount of Cd increase was related to: 1) the concentration of Cd in the diet; 2) the Cd source (Ft. Collins sludge vs. Metro Denver sludge), and 3) the time period of sludge ingestion. Copper, Pb, and Cd in liver tissues were shown to increase in a linear fashion during sludge ingestion. Cadmium did not decrease in liver or kidney tissues when sludge was removed from the diet. Persistent organics were concentrated in fat tissues of cattle that consumed sewage sludge. The effects to cattle from exposure or ingestion of sewage sludge appear benign. Thus, the principal health hazards associated with grazing cattle on sludge fertilized pastures would be the possibility of increased levels of heavy metals entering the human food chain through kidney and liver consumption. Additional studies were conducted measuring the die-off rate of pathogens in liquid sludge as it dried in earthen drying basins. The number of fecal coliforms, total coliforms, salmonellae, f2 and T7 bacteriophages, and Ascaris ova decreased rapidly as the sludge dried.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Sludge, sewage, disposal, kidney, liver fat, bone, muscle, cattle, trace metals, trace elements, zinc, copper, nickel, cadmium, lead, forages, soil, organics, PCB's, sludge ingestion, pathogens.	Heavy metals, toxic elements, trace metals, persistent organics, micronutrients, sewage sludge, health effects, sludge disposal, sludge ingestion, animal health, food chain	
19. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 172
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTION
HERE

DISCLAIMER

The information in this document has been funded wholly or in part by the United States Environmental Protection Agency, and the Office of the Associate Director for Physical Science, U.S. Food and Drug Administration, under Contract No. 68-03-2210 to Metropolitan Denver Sewage Disposal District No. 1. It has been subject to the Agency's peer and administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

6 1/2"

8 1/8"

BEGIN
LAST LINE
OF TEXT

3/8"

ii

PAGE NUMBER

BOTTOM OF
(IMAGE AREA)
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from drinking water supplies, and to minimize the adverse economic, social, health and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

The Federal Water Pollution Control Act in 1972 (P.L. 92-500) mandated that wastewater be treated to acceptable levels in order to reduce, prevent and eliminate water pollution. As a result of this sweeping legislation and subsequent legislation such as the Clean Water Act of 1977 (P.L. 95-217), the Nation is producing and will produce tremendous quantities of wastewater residuals (sludge). The disposal or use of this sludge is a national environmental problem that is two-fold: the sludge must be handled in a cost effective and environmentally safe manner. Because the use of the sewage sludge on agricultural land can meet these requirements, it has received special attention. But concerns exist over environmental food chain effects of pathogens, toxic metals, pesticides, and persistent organics such as PCB's. The research described in this report attempts to answer some of these questions and concerns.

Francis T. Mayo, Director
Municipal Environmental
Research Laboratory

ABSTRACT

This research was initiated to examine the effects of sewage sludge disposal on agricultural land and potential for transmission of sludge contaminants into the food chain. Soils, forages and cattle at a sewage sludge disposal site were examined for trace metals and persistent organics. Soils on the disposal site had increased concentrations of Zn, Cu, Ni, Cd and Pb; however, these metals were concentrated in the plow layer with no indication of leaching below the depth of sludge incorporation. Forages at the disposal site had higher levels of Zn, Cd, Cu and Ni than nearby control (no sludge applications) forages. But the lead contents of sludge-grown forages were significantly lower than control forages.

Cattle grazing on the sludge disposal site were healthy with no signs of pathology. The sludge-exposed cattle showed higher concentrations of Cd in kidney tissue when compared with one group of younger unexposed control animals; but no significant difference was noted when they were compared with a larger group of unexposed animals of known age. Cadmium concentrations of kidney tissues were shown to increase significantly with the age of the cattle. Persistent organics in tissues of cattle grazing on the sludge disposal site were not elevated over levels in unexposed cattle.

Cattle that were fed sewage sludge as a percentage of their diet (to simulate grazing) remained healthy. The sludge showed no positive or negative effects on cattle performance other than to act as a diet diluent. Young steers were fed a feed lot ration consisting of approximately 0%, 4%, and 12% anaerobically digested Metro Denver sludge for 94 days. Cattle consuming the sludge amended diets showed increased concentrations of Pb, Hg, As, and Cd in kidney. Cadmium in the kidney increased from 1.1 to 2.5 ppm (dry weight) from the ingestion of the 4% sludge diet; but no further increase was seen when the cattle ingested a 12% sludge diet. Concentrations of Cu and Mo in kidney decreased, whereas, Zn, Se, and Ni were unaffected by the sludge ingestion. Concentrations of Zn, Cd, Cu, Pb, Hg, and As significantly increased in liver when the 12% sludge diet was consumed, whereas Mo decreased and Ni and Se were unaffected. Arsenic and Hg increased slightly in muscle, but other metals showed no changes.

Young steers were fed a feedlot ration consisting of 0% and 12% anaerobically digested Ft. Collins sewage sludge for 106 days. The Fort Collins sludge contained 5 times as much Cd and more than twice as much Cu as the Metro Denver sludge. Kidney levels of Cd increased from 1.2 to 14 ppm. The ingestion of the Fort Collins sludge significantly increased liver concentrations of Cd, Pb, and Hg, but Zn concentrations were not affected. Liver-Cu concentrations were not increased, despite the increased dietary Pb, and Cu. Mercury increased slightly in muscle tissue, but other metals showed no changes.

Cows and young steers were fed a diet containing 0% and 12% anaerobically digested Fort Collins sewage sludge for 9 months, which was followed by a 4 month withdrawal period in which no sludge was fed. Cadmium, Pb, and Cu increased significantly in the liver and kidney during the 9 month period. Analyses of liver biopsies taken at intervals throughout the study showed that uptake of Cd, Cu and Pb into liver was linear with time. Cadmium increased from approximately 1 to about 20 ppm during the 9 month period. Lead and Cu increased in liver tissues during the sludge ingestion period; however, both decreased in a linear pattern when sludge was removed from the diet. There was no significant decrease in Cd concentration in the liver during the sludge withdrawal period. Other trace metals (Zn and Fe) were not affected by the sludge ingestion. Kidney concentrations of Cd increased from approximately 5 to 55 ppm during the sludge ingestion period, with no decrease when sludge was removed from the diet. Lead levels in the kidney increased during the sludge ingestion period, whereas Zn, Fe, and Cu were not significantly affected. Muscle tissues showed a slight but significant increase in Cd concentrations during the sludge ingestion period. Cows showed no significant decrease in muscle Cd after the sludge withdrawal period, but muscle Cd in steers did decrease significantly. Lead concentrations of bone increased due to the sludge ingestion, and did not decrease after the withdrawal period. Other metals in bone were not affected.

Body retention of Cd from the ingested sewage sludges was estimated to be less than 1%, which was less than the 2 to 9% reported by other researchers using Cd salts and other species of animals.

Fat tissue from all sludge ingestion studies showed increases in PCB's, DDE, dieldrin and oxychlordane. DDE and dieldrin decreased significantly when sludge was removed from the diet. PCB's increased from trace levels to approximately 4 ppm after 9 months of sludge ingestion. There were slight decreases of PCB's (not significant) after sludge was removed from the diet.

The effects on cattle from exposure or ingestion of sewage sludge appear to be benign. Thus, the principal hazards associated with grazing cattle on sludge fertilized pastures would be by increased levels of Cd entering the human food chain through kidney and liver consumption. Because of the low levels of heavy metals and persistent organics in the Metro Denver sludge and the fact that the sludge was plowed under in calcareous soils, the exposure of grazing cattle to these sludge-borne contaminants was effectively reduced.

Studies were conducted examining the die-off rate of pathogens in liquid sludge as the sludge dried in earthen drying basins. Analysis showed that as the sludge dried, the number of fecal coliforms, total coliforms, salmonellae, f2 and T7 bacteriophage, and ascaris ova decreased to low levels within periods ranging from weeks to months.

This report was submitted in partial fulfillment of Contract No. 68-03-2210 by the Metropolitan Denver Sewage Disposal District No. 1 under the sponsorship of the U.S. Environmental Protection Agency and the U.S. Food and Drug Administration. This report covers the period June 1975 to January 1980 and work was completed as of February 1982.

Cows and young steers were fed a diet containing 0% and 12% anaerobically digested Fort Collins sewage sludge for 9 months, which was followed by a 4 month withdrawal period in which no sludge was fed. Cadmium, Pb, and Cu increased significantly in the liver and kidney during the 9 month period. Analyses of liver biopsies taken at intervals throughout the study showed that uptake of Cd, Cu and Pb into liver was linear with time. Cadmium increased from approximately 1 to about 20 ppm during the 9 month period. Lead and Cu increased in liver tissues during the sludge ingestion period; however, both decreased in a linear pattern when sludge was removed from the diet. There was no significant decrease in Cd concentration in the liver during the sludge withdrawal period. Other trace metals (Zn and Fe) were not affected by the sludge ingestion. Kidney concentrations of Cd increased from approximately 5 to 55 ppm during the sludge ingestion period, with no decrease when sludge was removed from the diet. Lead levels in the kidney increased during the sludge ingestion period, whereas Zn, Fe, and Cu were not significantly affected. Muscle tissues showed a slight but significant increase in Cd concentrations during the sludge ingestion period. Cows showed no significant decrease in muscle Cd after the sludge withdrawal period, but muscle Cd in steers did decrease significantly. Lead concentrations of bone increased due to the sludge ingestion, and did not decrease after the withdrawal period. Other metals in bone were not affected.

Body retention of Cd from the ingested sewage sludges was estimated to be less than 1%, which was less than the 2 to 9% reported by other researchers using Cd salts and other species of animals.

Fat tissue from all sludge ingestion studies showed increases in PCB's, DDE, dieldrin and oxychlorane. DDE and dieldrin decreased significantly when sludge was removed from the diet. PCB's increased from trace levels to approximately 4 ppm after 9 months of sludge ingestion. There were slight decreases of PCB's (not significant) after sludge was removed from the diet.

The effects on cattle from exposure or ingestion of sewage sludge appear to be benign. Thus, the principal hazards associated with grazing cattle on sludge fertilized pastures would be by increased levels of Cd entering the human food chain through kidney and liver consumption. Because of the low levels of heavy metals and persistent organics in the Metro Denver sludge and the fact that the sludge was plowed under in calcareous soils, the exposure of grazing cattle to these sludge-borne contaminants was effectively reduced.

Studies were conducted examining the die-off rate of pathogens in liquid sludge as the sludge dried in earthen drying basins. Analysis showed that as the sludge dried, the number of fecal coliforms, total coliforms, salmonellae, f2 and T7 bacteriophage, and ascaris ova decreased to low levels within periods ranging from weeks to months.

This report was submitted in partial fulfillment of Contract No. 68-03-2210 by the Metropolitan Denver Sewage Disposal District No. 1 under the sponsorship of the U.S. Environmental Protection Agency and the U.S. Food and Drug Administration. This report covers the period June 1975 to January 1980 and work was completed as of February 1982.

CONTENTS

Foreword	iii
Abstract	iv
Figures	ix
Tables	xi
Acknowledgment	xv
1. Introduction	1
2. Conclusions	4
3. Recommendations	9
4. Background	10
a) Arsenic	10
b) Cadmium	11
c) Copper	13
d) Molybdenum	13
e) Fluorine	14
f) Lead	15
g) Mercury	15
h) Nickel	15
i) Selenium	16
j) Zinc	17
k) Organics	18
l) Effects of sludge ingestion on livestock	20
m) Pathogens	24
n) Sludge disposal and chemical composition of Metro Denver's sewage sludge	25
Chemical constituents of Metro Denver's sewage sludge	25
5. Experimental Procedures	29
a) Soil and plant survey of Lowry Bombing Bombing and control ranch	29
b) Health and trace element levels in beef tissues from cattle grazing sludge amended soils at the Lowry Bombing Range	31

FROM
PAGE
LINE OF
TEXT
HERE

TABLE OF CONTENTS

CHAPTER
PAGE

FROM
PAGE
LINE OF
TEXT
HERE

c)	Investigation of heavy metal content in kidney and liver tissues from cattle of known age and no previous exposure to elevated levels of heavy metals	32
d)	Ingestion of Metro Denver sewage sludge by beef cattle	33
e)	Ingestion of Ft. Collins sewage sludge by beef cattle	37
f)	Ingestion of Ft. Collins sewage sludge by beef cattle 9-month ingestion period	41
g)	Pathogen survival during air drying of digested sludges.	42
6.	Results and Discussion	45
a)	Soil and plant survey of LBR and control ranch	45
	Heavy metals and nutrients in soils and forages at the LBR and control ranch	45
	Refractory Organics at the LBR and control ranch	54
b)	Health and trace element levels in beef tissues from grazing sludge amended soils at the LBR	56
	Estimate of soil consumption by LBR cattle	56
	Health and pathological examination of beef cattle grazing sludge amended soils at the LBR	56
	Concentration of trace metals in tissues of range cattle grazing sludge amended soils at the LBR	57
	Refractory organic residues in tissues of cattle grazing the LBR and control ranch	61
c)	Investigation of heavy metals content in kidney and liver tissues from cattle of known age and no previous exposure to elevated levels of heavy metals	61
d)	Effects of direct ingestion of Metro Denver sludge by beef cattle	65
	Performance of cattle fed Metro Denver sludge	65
	Trace element concentration of beef cattle tissues fed Metro Denver sewage sludge	67
	Refractory organic residues in tissues of beef cattle fed Metro Denver sewage sludge as a portion of their diet	72
e)	Effects of direct ingestion of Ft. Collins sewage sludge by beef cattle - 3 month ingestion period	73
	Performance of cattle fed Ft. Collins sewage sludge	73
	Trace element concentration of tissues from beef cattle fed Ft. Collins sewage sludge	75
	Some comparisons between the Ft. Collins and Metro Denver feeding trials in relation to heavy metal content of cattle tissues	76

FROM
PAGE
LINE OF
TEXT
HERE

FROM
PAGE
LINE OF
TEXT
HERE

FROM
PAGE
LINE OF
TEXT
HERE

FIGURES

<u>Number</u>	<u>Page</u>
1 Location of study area	29
2 Mean concentration of Cd in kidney tissues as a function of cattle age	64
3 Mean concentration of Cu in liver tissues as a function of cattle age	65
4 Cadmium concentrations (dry weight) of liver biopsies during 9 month sludge ingestion period and 4 month withdrawal period . .	88
5 Copper concentrations (dry weight) of liver biopsies during 9 month sludge ingestion period and 4 month withdrawal period . .	88
6 Lead concentrations (dry weight) of liver biopsies during 9 month sludge ingestion period and 4 month withdrawal period . .	89
7 Lead concentrations of bone biopsies during 9 month sludge ingestion period and 4 month withdrawal period	89
8 Sludge moisture content, average weekly high and low ambient temperatures, and precipitation	94
9 Survival of f2 indicator virus, total coliform, fecal coliforms, and salmonellae in anaerobically digested sludge in drying basin 1 for the period of 13 January to 11 July 1977	95
10 Experiment 2 - Sludge moisture, average daily high and average daily low temperatures and precipitation. Up to week 30 ADH, ADL, and precipitation are shown on weekly basis, after week 30 they are shown on a monthly basis	96
11 Survival of f2 indicator virus, total coliforms, fecal coliforms, and salmonellae in anaerobically digested sludge for the period of April 20, 1977 to October 8, 1978	96
12 Sludge moisture, average daily high and low ambient temperatures, and precipitation for the period July 15, 1977 to January 5, 1978	97
13 Survival of f2 indicator virus, total coliforms, and fecal coliforms in anaerobically digested sludge for the period of July 15 to January 5, 1978	97
14 Average weekly high and low ambient temperatures and precipitation for Experiment 4	98

Number

Page

- | | | |
|----|---|-----|
| 15 | The moisture content of anaerobically digested sludge of
Experiment 4, March 29 to August 19, 1978 | 98 |
| 16 | Survival of bacteriophages in drying sludge of Experiment 4 . . . | 99 |
| 17 | Survival of total and fecal coliforms and salmonellae in
drying sludge of Experiment 4 | 100 |

TABLES

<u>Number</u>		<u>Page</u>
1	Pesticides and PCB Content of Sewage Sludges	18
2	Heavy Metal Content of Kidney and Liver Tissues from Cattle that Have Ingested Various Amounts of Different Sewage Sludges . . .	22
3	Chemical Composition of Metro Denver's Sludge for the Years 1975 and 1976	27
4	Mean Heavy Metal Content of Metro Denver's Anaerobically Digested Primary Sewage Sludge	28
5	Concentration of Refractory Organic Residues in Sewage Sludges, Influent, and Effluent Waste Waters	28
6	List of Organic Residues that were Analyzed For	31
7	Ingredients of Pelleted Basal Concentrate Mix	33
8	Amount of Diet Components Consumed by Treatment Cattle During the Metro Denver Sludge Feeding Trial	34
9	Percent Ash, Dry Matter, Protein, and Fiber Content of Pellets Fed to Treatment Cattle	34
10	Mean Trace Element Composition of Metro Denver Sewage Sludge and Diet Components Added to the Diets of Cattle	35
11	Trace Element Composition of Diets Amended with Metro Denver Denver Sewage Sludge	36
12	Concentration and Range of Residues in Sludge, Silage, Feed, and Water Used in Metro Denver Sludge Feeding Trial	37
13	Amount of Diet Components Consumed by Cattle During the Ft. Collins Sludge Feeding Trial	38
14	Percent Ash, Crude Protein, Acid Detergent Fiber, and Ash-Free Acid Detergent Fiber of Pellets	38
15	Mean Concentration of Heavy Metals Contained in Metro Denver Sludge, Ft. Collins Sludge, and Diet Components of Ft. Collins Feeding Trial	39
16	Concentration of Zn, Cd, Cu, Hg, and Pb of Diets Amended with Ft. Collins Sludge	40
17	Mean Concentration of Refractory Organics Determined in Sludge, Feed, and Silage	40

<u>Number</u>		<u>Page</u>
18	Selected Chemical Constituents of Soil From the Control Ranch and Soil From the LBR Which had Never Received Sludge Applications	45
19	Mean and Range of Total Heavy Metal Content of Surface Soils From the Control Ranch and LBR Which had Never Received Sludge Applications	46
20	Mean and Range of Heavy Metals of Forage Tissues From Unsludged Areas Within the LBR and Control Ranch	46
21	Mean, Median, and Range of Heavy Metals in Surface Soil Samples From Areas of Sludge Application and Control Areas Within the LBR	47
22	Composition of LBR Surface Soils From Areas of Sludge Application and Areas That Have Not Received Sludge	48
23	Median Concentration and Range of Nitrates and Phosphorus With Soil Depth at the LBR	49
24	Concentration of Total Soil Zn, Cd, Cu, Ni, and Pb With Soil Depth From an Unsludged Area of the LBR	49
25	Concentration of Total Soil Zn, Cd, Cu, Ni, and Pb After 295 mT/HA of Sludge Had Been Applied	50
26	Heavy Metal Concentrations of Forages From the LBR	50
27	Correlation Coefficients of DTPA Soil Extractable Zn, Cd, Cu, Ni, and Pb Compared With the Metal Uptake by Forages	51
28	Correlation Coefficients of Total Soil Zn, Cd, Cu, Ni, and Pb Compared With the Metal Uptake by Forages	52
29	Correlation Coefficients of DTPA Soil Extractable Zn, Cd, Cu, Ni, and Pb compared With the Total Soil Metals	52
30	Concentration of Total Soil Zinc Compared to Expected Concentrations After Known Sludge Additions	53
31	List of Organic Residues Identified in Sludge or Soil Samples	54
32	Concentrations of Refractory Organics Residues Determined in Sludge Types Disposed of at the LBR	55
33	Mean Concentration of Trace Elements in Tissues of Sludge Exposed LBR Range Cattle and Control Ranch Cattle	58
34	Concentration of Refractory Organic Residue Determined in Fat Tissues of Range Cattle From the LBR and Control Ranch.	61
35	Age Data of Dairy and Range Cattle Having Never Been Exposed to Sewage Sludge Which Were Used in the "Animal Age" Study	62

<u>Number</u>		<u>Page</u>
36	Mean Concentration of Trace Metals Determined in Kidney and Liver Tissues from Control Cattle of Known Age	62
37	Mean Tissue Concentration of Control Range Cattle and Sludge Exposed LBR Cattle	63
38	Amount of Feed and Sludge Consumed and the Overall % Feed Efficiency From the Sludge Treatments (Metro Denver Sludge) . .	66
39	The Effect of Sludge (Metro Denver) in Diet Upon Feed Lot Cattle Performance	66
40	Mean Concentration of Zn, Cd, Cu, Pb, and Hg in Kidney and Liver Tissues of Beef Cattle Fed a Diet Containing 0, 4, and 12% Metro Denver Sludge for 94 Days	67
41	Mean Concentration of As, Mo, Ni, and Se in Kidney and Liver Tissues of Beef Cattle Fed a Diet Containing 4 and 12% Metro Denver Sludge for 94 Days	68
42	Mean Concentrations of Zn, Cd, Cu, Pb, Hg, and F in Bone, Blood and Muscle Tissues of Beef Cattle Fed 0, 4, and 12% Metro Denver Sludge	69
43	Mean Concentrations of As, Mo, Ni, and Se in Bone, Blood, and Muscle Tissues of Beef Cattle Fed 0, 4, and 12% Metro Denver Sludge	70
44	Mean Concentration of Trace Elements in Spleen, Lung, Brain, and Fat Tissues of Beef Cattle Fed 0 and 12% Metro Denver Sludge. .	71
45	Range of Organic Residues Found in Sludge, Silage, Feed, and Water of Metro Denver Sludge Feeding Trial	72
46	Mean Concentration of p,p'-DDE, Dieldrin, and AR-1254 in Fat Tissues of Beef Cattle Fed Metro Denver Sewage Sludge	73
47	Concentration of Zn, Cd, Cu, Hg, and Pb in Sludge and Diets Amended With Ft. Collins Sewage Sludge	74
48	Amount of Feed and Sludge Consumed and the Overall Percent Feed Efficiency of Cattle Fed Ft. Collins Sludge	74
49	The Effect of Ft. Collins Sludge in Diets Upon Feed Lot Cattle Performance	74
50	Mean Concentration of Zn, Cd, Cu, Hg, and Pb in Kidney and Liver Tissues of Beef Cattle Fed a Diet Containing 0 and 12% Ft. Collins Sludge	75
51	Mean Concentration of Zn, Cd, Cu, Hg, and Pb in Tissues of Beef Cattle Fed a Diet Containing 0 and 12% Ft. Collins Sewage Sludge for 3 Months	76
52	Mean Cd, Zn, and Cu Tissue Concentration From Cattle Fed Ft. Collins or Metro Denver Sludge	77

<u>Number</u>		<u>Page</u>
53	Copper Concentrations in Kidney and Liver Tissues of Beef Cattle Fed a Diet Containing 0 and 12% Metro Denver or Ft. Collins Sludge	78
54	Mean Weight of Diet Consumed Per Animal, the Concentration of Cd and Pb in the Total Diet, and the Amount of Cd and Pb Consumed per Animal During the Treatment Period	79
55	Estimated Amount of Cd and Pb Contained in Kidney and Liver and Bone Tissues of Cattle Fed Diets of 0 or 12% Sewage Sludge	80
56	Mean Concentration of Refractory Organics Determined in Sludge, Feed and Silage	80
57	Mean Concentration of Chlorinated Hydrocarbons Within Liver Tissues	82
58	Mean Concentration of Chlorinated Hydrocarbons in Abdominal Fat of Beef Cattle Fed Sewage Sludge	82
59	Amount of Diet Components Consumed by Cattle During 9 Month Sludge Feeding Period	83
60	Elemental Concentration of Sludge and Diets Fed to Cattle During 9 Month Sludge Ingestion Period	84
61	Cow and Steer Weights During Ft. Collins Sludge Feeding Trial . .	84
62	Heavy Metals Concentrations In Kidney Tissues of Cattle After 9 Months of Sludge Ingestion and a 4 Month Withdrawal Period. . .	86
63	Heavy Metals Concentrations In Liver Tissues of Cattle After 9 Months of Sludge Ingestion and a 4 Month Withdrawal Period. . .	86
64	Heavy Metals Concentrations in Tarsal Bone Tissues of Cattle After 9 Months of Sludge Ingestion and a 4 Month Withdrawal Period. .	90
65	Heavy Metal Concentrations In Muscle Tissues After 9 Month of Sludge Ingestion and a 4 Month Withdrawal Period	90
66	Mean Concentration of Organic Residues Determined In Feed Components from 9 Month Sludge Ingestion Study	92
67	Average Concentration of Organic Residues Determined In Fat Tissue of Cattle Fed Ft. Collins Sludge	92
68	Length of Survival of f2, Fecal Coliforms, and Salmonellae in Sludge Drying Beds	101
69	Counts of Ascaris Ova and Percent Active Ascaris in Sludge of Experiment 4	101

ACKNOWLEDGMENTS

The authors of this report would like to acknowledge the many individuals who contributed their time and expertise to this project. We are particularly indebted to Mr. John Tessari of the Colorado Epidemiological Pesticide Studies Center, who directed the analysis of all refractory organics. Assays of many of the animal tissues were conducted by the Colorado State University Analytical Facility under the direction of Dr. William Ferguson, Dr. David Bigelow, and Dr. John Nichols. The antemortem, postmortem, and histopathological examination of all cattle were carried out by Dr. Rue Jensen and Dr. Darrell J. Schweitzer of the College of Veterinary Medicine, Colorado State University, Dr. William Halliwell of Wespeth Laboratories, Inc. Mr. Mario Aguilar, Ms. Kathryn Brown, Mr. Larry Bahr, Mrs. Barbara Millett and Mrs. Patti Skinner of the Metropolitan Denver Sewage Disposal District No. 1, are thanked for their many contributions in the analyses of soil, plant, sludge, and animal tissues, and for their efforts in the final preparation of this manuscript. Appreciation is also due to Dr. John Foss of the University of Maryland who helped with the description of the soil profiles at the Lowry Bombing Range and control ranch. Special thanks are extended to Dr. Rufus L. Chaney of the U.S. Department of Agriculture for his invaluable assistance in acquiring many of the references and for his helpful discussions throughout the project.

Last, a deep sense of gratitude is extended to Dr. George L. Braude of the U.S. Food and Drug Administration and Mr. Gerald Stern of the U.S. Environmental Protection Agency for their continuous guidance and helpful suggestions without which this project would have been impossible.

SECTION 1

INTRODUCTION

Sewage sludge is the residue or solid material that is removed from wastewater during treatment and purification. The handling and disposal of this sludge is one of the most problematic and costly processes within any wastewater treatment facility. Presently, approximately 50% of the sludge produced in the U.S. is applied to the land or landfilled; the remainder is either incinerated or dumped into the ocean. Increasing environmental concerns over air and water pollution will decrease the total amount of sludge incinerated or disposed of in the ocean. Thus, the percentage of sludge that will receive some sort of land disposal will most likely increase substantially in the future.

The idea of recycling waste materials to the land is certainly not new and has been an accepted agricultural practice since the inception of agriculture and civilization millennia ago. Sewage sludge contains a number of valuable nutrients and humus materials that can be used to increase soil fertility. The addition of organic matter to soil improves soil tilth, increases the cation exchange capacity, raises the water-holding capacity, and consequently may increase crop yield over that normally achieved through the use of conventional inorganic fertilizers.

Problems are associated with the recycling of sewage sludge. Much of our sewage is not only derived from garbage or fecal material but is the product of an industrial society. Modern society tends to centralize and concentrate industry, which is reflected in residues from wastewater treatment plants; thus, heavy metals and persistent organics such as PCB's (polychlorinated biphenyls) and pesticides, many of which are products or byproducts of industry, turn up in sewage sludge. In addition to these elements and compounds, there is a disease potential. The problems associated with many of these elements, compounds, and biological agents becomes particularly acute when they are potentially harmful to crops, animals or the human food chain.

The Metropolitan Denver Sewage Disposal District No. 1 has been recycling sewage sludge to land since 1969. The sludge has been applied to the soil surface, plowed under, and planted to forages such as winter wheat, oats sorghum, or sudan grass. These forages were subsequently grazed by a herd of beef cattle that had been on the sludge recycling site since the inception of sludge disposal there. The cattle grazing this land had been exposed to sewage sludge for a number of years, and had ample opportunity to ingest contaminants through translocation through plants or direct ingestion of soil and sludge. This situation represented a unique opportunity

to examine some long-term effects of sludge exposure to cattle from a somewhat typical ongoing practice.

The primary objective of this study was to examine whether and to what extent contamination of cattle tissue had occurred as a result of exposure to the sludge. To accomplish this objective, the project was divided into several separate studies. The first study involved a thorough survey of soils and plants at the Lowry Bombing Range (LBR) sludge recycling site to document the magnitude and variation of sludge contaminants. In conjunction with the soil and plant survey, a group of old range cows that had been at the LBR since the inception of sludge disposal and a group of range cows from a nearby control ranch were evaluated. The animals were slaughtered, and various tissues were analyzed for disease incidence and retention of trace elements and persistent organics.

Results of the tissue analyses of cows from the LBR indicated that concentrations of copper and cadmium from liver and kidney tissues were significantly different when compared with tissues of cattle from the control ranch. Veterinarian inspection of both groups of cattle at the time of slaughter revealed that the cattle from the LBR were significantly older than the control group of animals. The age difference between the two groups of animals had been unanticipated, and thus a separate age variable was introduced into the study. The introduction of this age variable made it impossible to determine whether the difference in tissue copper and cadmium concentrations were the result of sludge exposure or the result of normal aging processes. A separate study was undertaken to resolve the age variable.

Cattle with no previous exposure to sewage sludge, were examined to determine ranges and averages of heavy metals in kidney and liver tissue as a function of age.

Additional studies were designed around the premise that the application of sludge to soil or pasture land will result in the direct ingestion of sludge constituents. The direct feeding study would also resemble a situation in which sewage sludge is applied to forages and not plowed under and thereby a much greater ingestion of sewage sludge by cattle would be expected to occur. Three experiments were undertaken to examine the effects of direct sludge ingestion by cattle and to assess the magnitude of uptake of heavy metals and persistent organics into edible and other tissues of cattle.

The first direct feeding study was conducted with young steers and anaerobically digested Metro Denver sewage sludge. The sewage sludge was added to the diets of the steers at the rates of 0%, 4%, and 12% sludge (dry weight). The sludge was fed for a period of 3 months, after which the steers were slaughtered and tissue samples of kidney, liver, bone, muscle, blood, fat, lung, brain, and spleen were taken for trace metals and persistent organics analyses.

The first sludge feeding study was followed by a second feeding study in which an anaerobically digested sludge (Ft. Collins sludge), significantly higher in cadmium and copper, was fed at 0 and 12% sludge concentrations to

another group of cattle. This experiment enabled comparisons of the rate of heavy metal uptake and possible interactions of heavy metals within the sludges.

The third study again used Ft. Collins sewage sludge, but it was designed to examine depletion rates of organics and heavy metals from tissues after a long-term (9 month) sludge ingestion period. Periodic liver and bone biopsies permitted a more detailed investigation of uptake and depletion rates of tissue heavy metals. Uptake rates between old cows and young steers were also compared. Cattle from all of the studies were examined antemortemly and postmortemly by a veterinarian pathologist. In addition, tissue samples at slaughter were taken for histopathological examination.

Pathogens including enteric viruses, bacteria, protozoan cysts, and helminth ova have been shown to survive anaerobic digestion of sewage sludges, although the numbers of these organisms are usually greatly decreased if not eliminated by this process. Air drying in beds and storage in stockpiles has the potential for completely eliminating these organisms; but the time required needs to be evaluated.

To determine the time required for the destruction of human pathogens, anaerobically digested liquid sewage sludge was placed in earthen drying basins. The survival of endemic salmonellae, fecal coliform, inoculated ascaris ova, and inoculated f2 and T7 bacteriophages were followed until these indicator organisms could no longer be detected.

SECTION 2

CONCLUSIONS

Studies examining the trace element and refractory organic status of soils and plants at the Metro Denver sewage sludge recycling site showed that soil concentrations of zinc, copper, nickel, cadmium, lead, and mercury were increased and concentrations of arsenic, selenium, molybdenum, and 22 refractory organics were unaffected. Forage crops grown on these sludge amended soils showed increased plant tissue concentrations of zinc, copper, cadmium, and nickel; however, none of the metal concentrations were elevated to a phytotoxic level or above a range that would be considered normal for forage tissues. Concentrations of arsenic, selenium, and mercury in forage tissues were unchanged, and lead concentrations decreased as a result of sludge applications. None of the forage tissues examined contained detectable levels of refractory organics.

Cattle grazing the Metro Denver sludge disposal site (LBR) were healthy and did not have any greater incidence of disease or death loss than other herds in the area that had never been exposed to sludge. Postmortem pathological examination of internal organs showed no significant differences between the LBR cattle and a nearby control herd.

Heavy metal concentrations of tissues from cows grazing the sludge disposal site were compared with tissues of cows from a nearby control ranch where sludge had never been applied. The sludge-exposed cows had significantly higher levels of renal (kidney) cadmium and zinc, and significantly lower concentrations of hepatic (liver) copper. These tissue concentrations of copper, cadmium, and zinc were compared with concentrations of metals determined in kidney and liver tissues from another group of Colorado cows of known age and no exposure to sewage sludge. Comparisons demonstrated that tissue concentrations of zinc and cadmium may not have been increased as a result of sludge exposure, but may have been due to accumulation that occurs as cattle age.

Examination of control cattle showed that hepatic copper concentrations ranged from 9 to 285 ppm (dry weight) and did not appear to be related to age. Sludge exposed range cattle on the average contained lower concentrations of hepatic copper than non-sludge exposed control range cattle; however, this effect was most likely related to lower levels of dietary copper rather than to a sludge effect.

Young steers were fed a feedlot ration containing approximately 0%, 4% and 12% anaerobically digested Metro Denver sludge for 94 days. The steers

remained healthy and did not develop any signs of pathology. Steers consuming the sludge amended diets did not gain as much weight as the control steers. Expected gains of the 3 groups calculated from feed consumption agreed well with observed gains when the sludge component was assumed to have no energy value; thus, the anaerobically digested sludge acted as a diet diluent.

Trace element analysis of tissues from these sludge fed steers showed that the highest metal concentrations were found in kidney and liver tissues. Renal cadmium increased from 1.1 to 2.5 ppm (dry weight) when these steers consumed a 4% sludge diet, but no further increase was seen when the cattle ingested a 12% sludge diet. Therefore, cadmium absorption and retention was not entirely dose related. Renal concentrations of lead, mercury, and arsenic increased from ingestion of the 12% sludge ration, whereas, concentrations of copper and molybdenum appeared to decrease. Renal concentrations of zinc, nickel, and selenium were unaffected by the sludge additions.

Hepatic concentrations of zinc, cadmium, copper, lead, mercury, and arsenic significantly increased when the 12% Metro Denver sludge diet was consumed, whereas, molybdenum decreased and concentrations of nickel and selenium remained unchanged. Concentrations of lead, fluorine, and molybdenum increased in bone tissues due to sludge ingestion. Arsenic and mercury increased slightly in muscle tissues due to the ingestion of the 12% sludge diet, while other metals showed no changes. Blood levels of lead, molybdenum, and selenium increased, but other elements were unchanged. The only metal to show a significant change in spleen, lung, brain, or fat tissues was lead in lung tissues, which showed a slight increase due to sludge ingestion or inhalation.

Performance of steers fed feedlot rations containing 0% or 12% Fort Collins anaerobically digested sludge for 3 months was similar to that from feeding the Metro Denver sludge. All animals remained healthy with no signs of pathology, and the sludge was shown to have no positive or negative effects on the general performance of the cattle other than to act as a diet diluent.

The Fort Collins sludge, which contained 5 times as much cadmium and more than twice as much copper as the Metro Denver sludge, produced significant increases in renal cadmium, lead, and mercury. The renal cadmium level increased from 1.2 to 14 ppm (dry weight). Renal concentrations of zinc were not affected by the dietary sludge.

Hepatic concentrations of cadmium, lead, and mercury were significantly increased due to the ingestion of the Fort Collins sludge; despite the increased dietary copper, hepatic concentrations of copper were not increased as in the Metro Denver sludge feeding trial. Hepatic zinc concentrations were not affected. Lead concentrations were increased in bone tissues, but other elements were not affected. Lead concentrations also increased slightly in lung, brain, and spleen tissue. Cadmium increased in lung and spleen tissues, and mercury increased slightly in muscle, lung, and spleen tissues. Except for a slight increase of mercury, muscle tissues were unaffected by the ingestion of Fort Collins sludge.

Body retention of Cd from the ingested sewage sludges was estimated to be less than 1%, which was less than the 2 to 9% reported by other researchers using Cd salts and other species of animals.

The long-term ingestion (9 months) of Ft. Collins sewage sludge by cows and young steers produced no adverse effects on the general health and performance of the cattle. Comparisons of uptake and retention levels of heavy metals and persistent organics between the two groups of cattle revealed that there were no significant differences due to the age of the animals. Analyses of liver biopsies taken during the sludge ingestion period revealed that cadmium, lead, and copper concentration in liver tissues increased linearly throughout the sludge ingestion period. When sludge was removed from the diet, lead and copper in liver decreased at rates equivalent to the uptake rates; however, cadmium showed no significant decrease. Liver cadmium concentrations increased from approximately 1 to 20 ppm (dry weight) during the 9 month sludge ingestion period. Liver lead increased from approximately 1 ppm to 4 or 5 ppm (dry weight), but decreased to between 1 and 2 ppm during the 4 month sludge withdrawal period.

Bone biopsies showed increases of lead concentrations of 3 to 5 ppm due to the sludge ingestion, with no decrease during the withdrawal period. Other metals in bone were not affected by the sludge ingestion.

Cadmium concentrations in kidney of cows and steers significantly increased during the 9 month sludge ingestion period. The cadmium increase in cow kidneys was from 7.4 to 69 ppm (dry weight). The younger control steers had a significantly lower kidney cadmium level of (3.5 ppm) than the older control cows, but after 9 months of sludge ingestion, the average kidney cadmium concentration for the steers had increased to 64 ppm. Analyses of kidney tissues after sludge had been removed from the diet for 4 months showed no significant cadmium decrease. Lead and zinc concentrations in kidney from both groups of cattle increased after 9 months of sludge ingestion and did not decrease significantly when sludge was removed from the diet for 4 months. Iron and copper in kidney tissues were not significantly affected by the sludge ingestion.

Muscle tissues showed a significant cadmium increase after 9 months of sludge ingestion. The cadmium content of muscle tissues from cows increased from 0.12 to 0.27 ppm (dry weight); whereas, cadmium in steer muscles increased from 0.14 to 0.43 ppm. There was a significant muscle cadmium decrease in steers after sludge was removed from the diet, but no significant muscle cadmium decrease in the cows. Other metals (lead, copper, zinc, and iron) in muscle tissues were not affected by the sludge ingestion.

Persistent organics that were found to increase in fat tissues due to the ingestion of sewage sludge, were DDE, dieldrin, PCB's, TNC, HCB, and oxychlorthane. The only organics that accumulated to levels that could have a health significance were PCB's which increased approximately 6-fold from the dietary level (700 ppb) to the level found in fat tissues (4200 ppb)

during the 9 month sludge ingestion period.

The cattle grazing the LBR sludge disposal site did not have elevated levels of persistent organics in fat tissues, nor were there detectable levels of organics in the soils or forages. This would indicate that there should be little to no hazard to the food chain from persistent organics if sewage sludges are incorporated into the soil. Uptake of persistent organics from the direct sludge ingestion studies indicated that PCB's could represent a hazard if significant amounts of sludge are consumed for extended periods of time or if high concentrations are present in the sludge. The 9 month sludge ingestion studies tend to agree with the work of Harrison et al. (1970) which showed that the steady state residue concentrations of DDE and DDT in fat tissues were about 5 to 6 times the concentration in the diet.

In relation to trace metals contaminants and persistent organics in cattle from the LBR sludge disposal site, it is important to note that the Metro Denver sludge, which was relatively low in heavy metals and persistent organics concentrations to begin with, was also plowed into calcareous soils. These conditions greatly limited the amount of sewage sludge exposure to the grazing cattle (as compared to cattle directly fed sewage sludge as part of their diet) and thereby probably limited the opportunities for direct ingestion of sewage sludge contaminants.

From these studies it can be seen that the ingestion of high levels of sewage sludge by cattle raises the heavy metal concentrations of a number of body tissues. However, muscle tissue, the principal food tissue for man, was effectively screened from excessive accumulations of the metals examined in this study. The entry of cadmium and other heavy metals into the diet of man would be largely through liver consumption. While high levels of cadmium were reached in liver tissues of cattle consuming Ft. Collins sludge for 9 months, this is an extreme case, and is not likely to occur under normal agricultural practices. Further, the cadmium concentrations of liver tissues from cattle fed the two sludges for a 3 month period were not significantly higher than cadmium concentrations found in the older unexposed range cows. Thus, it appears that cattle would act as an effective screen against these metals if sludges are used for the fertilization of agricultural lands. If low metal sludges, such as the Metro Denver sludge, are applied to calcareous soils as a fertilizer, and subsequently tilled into the plow layer, there would appear to be little potential hazard to the food chain.

Regulators have been particularly concerned with cadmium entry into the food chain; thus, regulations have concentrated on cadmium with little concern for other trace metals such as zinc, copper and iron. The work in this report and research conducted by others has shown that these metals interact and should not be considered separate entities. When sludge is applied to soils or ingested by animals, zinc, copper and iron may also increase; thus the bioavailability and toxicity of cadmium is changed. The studies discussed in this report have shown that the cadmium contained in sewage sludge is not as available for absorption and retention into animal tissues as other animal studies have shown utilizing additions of various chemical compounds of cadmium.

Microbiological studies showed that with adequate drying and residence time in earthen drying beds, any enteric pathogens remaining in the sludge after anaerobic digestion should be destroyed. The virus indicator organism f2 had a maximum survival time of 19 weeks even though initial concentrations were orders of magnitude in excess of any enteroviruses. Salmonellae and fecal coliform bacteria persisted for 70 and 73 weeks respectively, because of their ability for regrowth. Sludge desiccation may have been the most important factor in bringing about the disappearance of these persistent organisms. Salmonellae were not detectable after the sludge moisture concentration decreased to 30%; however, fecal coliforms were still detectable at less than 5% moisture concentration but at very low densities.

Ascaris ova also are sensitive to moisture. Only a small portion of the inoculated ova were found when the sludge moisture concentration decreased to 7.7%. Since previous studies had shown that the Nile-blue test overestimates the number of viable ova, it is unlikely that any viable ova remained in the inoculated sludge after the moisture content decreased to 10% or less.

The storage of sludge for a year plus a decrease in sludge moisture concentration to 10% or less should reduce the densities of any pathogenic organisms left after anaerobic digestion to undetectable levels. The likelihood of pathogenic microorganisms moving from the sludge into groundwater before being inactivated is negligible.

SECTION 3

RECOMMENDATIONS

- The bioavailability of cadmium from different food sources needs to be evaluated. The research described here has indicated that cadmium contained in sewage sludges is less available for absorption and retention into animal tissues than previously shown utilizing chemical salts of cadmium. Other research has shown even lower retention levels utilizing plant materials. The bioavailability from different crops (grain versus leafy tissues) into various animal species needs to be investigated for a more accurate measure of the real or perceived hazards of cadmium.
- Sewage sludge concerns have centered around the cadmium issue, with little regard for other trace metals. However, when sewage sludge is applied to soils or ingested by animals, potential toxic effects of cadmium are most likely mitigated by other trace metals such as zinc, copper or iron. Additional research is needed to evaluate possible interactions of cadmium with other trace elements that could affect cadmium uptake in animal tissues.
- Cadmium has been implicated as a casual agent in a number of human health problems. However, except for obvious industrial exposure, detrimental health effects of cadmium in the food chain have not been well documented. Epidemiological studies need to be carried out relating cadmium in the food supply to renal tubular dysfunction and related increase in urinary β_2 -microglobulin. The chemical significance of this protein should also be determined. Also, the validity of the 200 ppm cadmium in human renal cortex as a critical concentration, from which many regulations are based, should be investigated.
- Considerable evidence exists to show that sewage sludges (including anaerobically digested sludges) contain viral, bacterial, protozoan and helminth (parasites) pathogens. The paucity of data relating disease outbreaks to the specific use of anaerobically digested sludges would suggest that these sludges present little hazard to grazing livestock or workers. However, there are still questions as to how long land treated with anaerobically digested sludge or partially disinfected sludge should remain out of vegetable crop production; or how long cattle should be kept off pastures to ensure the health of livestock and the general public. The research reported here has shown ascaris ova may remain viable for long periods of time. Whether these ova could represent a significant health hazard, and for what period of time, needs to be verified.

SECTION 4

BACKGROUND

The application of municipal sewage sludge to agricultural land can be desirable from several aspects. The more obvious benefits are that nitrogen and other plant nutrients are recycled back to the soil instead of being generated using non-renewable resources. Sewage sludges also contain trace elements (i.e., zinc, copper, molybdenum, manganese, iron and boron) which, when used properly, are valuable plant micro-nutrients. Many soils which are deficient or marginally sufficient in these elements can be significantly improved by the application of sewage sludges.

The problems in utilizing sewage sludge as an agricultural fertilizer are diverse and complex. One of the more pressing problems is the question of heavy metals. There is concern that indiscriminate disposal of sewage sludges on agricultural lands may lead to decreased crop yields and/or may endanger the human food chain through excessive accumulations of some of these metals in edible portions of crops. There is also a potential health hazard to domestic livestock which graze sludge treated pastures. Healy (1968, '70, '72, '74) has shown that grazing livestock consume significant quantities of soil; one study estimated that when liquid sludge was applied to pastures, as much as 3% of the dry matter consumed by cattle (in fescue pastures) was sludge that adheres to the grass even after a number of rainfalls (Anonymous, 1977, 1979 b). Thus, cattle grazing sludge-treated lands are exposed to varying amounts of ingested sludge.

When sewage sludge is applied and mixed with the soil, many of the potentially hazardous metals are rendered insoluble and are unavailable for plant uptake; consequently, they pose little threat to animals that consume these crops. However, if sludge is ingested directly, metals that are innocuous in the soil may become potentially detrimental. Each of the elements examined in this study (arsenic, cadmium, copper, fluorine, mercury, molybdenum, nickel, lead, selenium and zinc) have been considered potentially harmful, either to crop or animals.

ARSENIC

Arsenic is a metalloid that has generated considerable interest because of its many compounds that have toxic properties. Arsenic in the environment in relation to arsenical pesticides, soil absorption, plant uptake and the food chain has been reviewed by Woolson (1975).

Sewage sludges generally contain low concentrations of arsenic, ranging from one to several ppm (dry weight). However, a few sludges may contain as much as 1,000 ppm (CAST, 1976). The chemistry of arsenic closely resembles that of phosphorus. Arsenate, which is not readily available for plant uptake, is the dominant chemical species of arsenic in soils. Arsenic tends to accumulate in the roots of plants; thus, little is transported to edible portions of crops. If the edible portion of a crop does not include the root, tuber, or whole plant, it is unlikely that tolerance levels for arsenic (2.6 ppm wet weight) (U.S. Department of Agriculture, 1968) would be exceeded even where arsenic phytotoxicity reduced growth by 50% (Woolson, 1973, 1975).

Arsenicals have been used as feed additives in poultry and swine diets for over 30 years. While arsenic is readily absorbed into animal tissues (particularly kidney and liver) it is also rapidly eliminated. Calvert (1975) found that when a diet containing 273.3 ppm arsenic (dry weight) as arsenilic acid was fed to sheep for 28 days, the liver arsenic increased from <0.01 to 29.2 ppm arsenic (dry weight). However, liver arsenic concentrations dropped to 5.0 ppm (dry weight) when the arsenilic acid had been removed from the diet for 6 days. This level is below the tolerance level of 2.0 ppm (wet weight) that the U.S. Food and Drug Administration has established for livers of marketable poultry and swine that have had arsenicals added to their diets.

Because arsenic concentrations tend to be low in sludges and are not readily available for plant uptake or transport to edible plant tissues, the hazards from arsenic in sludge applied to agronomic land should be minimal.

CADMIUM

Cadmium has been linked to a number of human health problems which includes hypertension, renal dysfunction, and Itai Itai disease. Cadmium is cumulative and may lead to health problems at relatively low concentrations. It can enter the human body by a number of pathways which include food, water, and through respiration of air containing cadmium dust. It has been postulated that any increase in the human body burden of cadmium may be detrimental. For these reasons, cadmium is considered to represent the most serious health problem from sludge application.

A major emphasis in regulating or controlling sludge applications to agronomic soils has revolved around the cadmium problem. Recommendations as to amounts of sludge that can be applied over a period of years to agricultural soils are aimed at limiting the amount of cadmium available for plant uptake. Various methods for accomplishing this are: to limit the sludge cadmium content; to limit the agronomic use of sewage sludge to only those sludges which contain low levels of cadmium; limit the annual and/or total amount of cadmium applied to the soil; maintain the soil pH at 6.5 or above, which keeps cadmium relatively insoluble and unavailable for plant uptake; or to grow non-edible crops, i.e., trees, turf-grass, etc. For a more complete discussion of how to limit uptake of cadmium see the CAST

report, 1976; Chaney, 1973, '75, '76, '77; and Pahren et al., 1977.

Cadmium (Cd) levels in municipal sewage sludges range from 3 to 3,000 ppm (dry weight basis) with a median value of 16 ppm (CAST, 1976). Cadmium is a ubiquitous element which is found in low concentration in most soils. Soil concentrations from 0.01 to 7 ppm are typical with an average value of about 1 ppm (Allaway, 1968; OECD, 1975). Additions of sewage sludge to agricultural land will raise the cadmium level of these soils, but with proper management these additions can be kept down to reasonable levels with little increase in plant uptake of cadmium.

The subjects of cadmium in the environment, food chain and animal nutrition have recently been reviewed by several authors (Fox, 1976; OECD, 1975; Friberg et al., 1974; Doyle, 1977; Perry, 1976; Petering, 1974; Piscator, 1976; Neathery, 1975; Fulkerson et al., 1973). Cadmium accumulates in the kidney and liver (and to a lesser extent in the pancreas, spleen and small intestine) of animals where it is bound by a protein called metallothionein. Studies have shown that as the dietary intake of cadmium increases, the concentration of cadmium in the kidney and liver also increases; however, there is only a slight increase in other tissues such as muscle or fat (Doyle et al., 1974; Decker et al., 1958; Schroeder, 1962; Friberg et al., 1974). The kidney carries approximately one-third of the total body burden of cadmium. Normal cadmium concentration in the renal cortex of adult humans with no known occupational exposure varies from 25 to 100 $\mu\text{g/g}$ wet weight or approximately 100 to 500 $\mu\text{g/g}$ on a dry weight basis (OECD, 1975). Experiments with animals including human subjects tend to indicate that from 2 to 6 percent of ingested cadmium is absorbed into body tissues (Friberg et al., 1974; OECD, 1975; McLellan et al., 1978; Flannagan et al., 1978). Friberg (1974) estimated the biological half life of cadmium to be over 30 years in humans; however, recent work of McLellan et al. (1978) and Flannagan et al. (1978) showed a biological half-life of 93 to 202 days.

Cadmium is known to interact with several other heavy metals, although the exact mechanisms are not as yet clear. Several investigators have noticed that increased exposure to cadmium has also increased zinc absorption (Bunn et al., 1977; Doyle et al., 1975; Webb, 1972; Banis et al., 1969). Friberg et al. (1974) postulated that this is probably due to the need for zinc to counteract the toxic action of cadmium. Cadmium may also act as a metabolic antagonist of copper. Copper storage in liver has been shown to be markedly reduced when cadmium was added to the diet of bovine, lambs, chickens and rats (Powell et al., 1964; Hill et al., 1963; Doyle et al., 1974, '75; Petering, 1974).

As noted earlier, the subject of cadmium is extremely complex. The available data are insufficient to realistically evaluate the behavior of cadmium in soils, plants and animals. Many questions remain to be answered concerning cadmium interactions with other heavy metals and their effects and relationships in natural food diets versus injury to animals throughout their life span. For the present, all that can be said is that cadmium may represent a potential hazard to the food chain, and caution should be exercised when sewage sludge is used for the production of food and feed crops.

COPPER

Copper is an essential element needed for plant and animal life. Many agronomic soils are deficient or marginally sufficient in copper; thus, moderate applications of sludge would in many cases improve crop response and quality. High soil copper concentrations can be toxic to plants, but the excess copper accumulates in the roots rather than being transported to aerial or edible parts of the plants. Copper does not build up to higher than normal levels in edible portions of the plant even under conditions of severe toxicity.

Under certain conditions animals can accumulate high concentrations of copper in the liver which can lead to liver damage. Copper toxicity is a common occurrence when sheep graze plants such as the *Heliotropium* species, which causes liver damage and subsequently prevents the homeostatic balance of copper. Copper toxicity in animals is also frequently caused by a copper-molybdenum imbalance and can be corrected by increasing the molybdenum content of the diet. High levels of dietary copper (250 ppm) balanced with zinc and iron have been used in swine rations because copper appears to act as an antibiotic (Underwood, 1977).

Because copper interacts with other elements such as zinc, iron, molybdenum and cadmium, it is difficult to state a toxic level of dietary copper based on copper alone. Suttle and Mills (1966) fed a diet containing up to 750 ppm copper (dry weight) to swine which produced severe toxicosis, but the toxicosis was eliminated when an additional 500 ppm zinc and 750 ppm iron were added to the diet. Sheep in Australia have been poisoned by grazing pastures on cupriferous soils where some plant tissues contained as much as 50-60 ppm copper (dry matter) (Underwood, 1977). Sheep have also succumbed to copper toxicosis when consuming fresh forages with normal copper levels (10-15 ppm) but extremely low molybdenum levels.

Sewage sludges vary widely in copper contents, ranging from 250 to 17,000 ppm (dry weight basis) (Chaney, 1973). Sheep are particularly sensitive to copper toxicity; thus, care should be taken when sheep are grazed on pastures that have been sprayed with liquid sewage sludge. Sheep should probably be kept off pasture until sludge is removed from the leaf surface by rainfall. Molybdenum is generally found in sludges in low concentrations; thus, molybdenum added with the sludge may offset possible copper toxicity to sheep. Presently, there is no evidence to indicate that ingestion of sewage sludge by sheep would be detrimental. Smith et al., (1977) fed sheep a diet containing 30% sludge for 69 days. This diet would have contained approximately 200 ppm copper (dry weight) and they found no evidence of toxicosis or increase in copper retention in kidney, liver or blood tissue.

MOLYBDENUM

Sewage sludges generally contain low concentrations of molybdenum. Sommers (1977) found a range from 5 to 39 ppm with a median of 30 ppm (dry

weight). While molybdenum is an essential element, it can cause molybdenosis or molybdenum induced copper deficiency in ruminants consuming fresh forage that is unusually high in molybdenum or low in copper. The dietary level of molybdenum required to produce molybdenosis varies with the copper and sulfate concentrations and with the animal species involved. Where copper concentrations in the diet are low (less than 4 ppm), levels greater than 20 ppm (dry weight) of molybdenum may lead to disturbed copper metabolism in ruminants (Underwood, 1977). Molybdenosis is caused by a copper-molybdenum imbalance and can be corrected by supplementing the diet with copper and phosphorus.

Molybdenum is an essential element for plants, but is required only at the ppm range for sufficiency. Molybdenum toxicity to crops is apparently not a problem since plant levels of a few hundred ppm are not toxic. Molybdenum is more available for plant uptake and removal from the soil profile through leaching under calcareous soil conditions than in acid soils. Because sewage sludges contain low concentrations of molybdenum that are accompanied with copper and phosphorus there is little concern that molybdenum in sludges would constitute a hazard to grazing animals.

FLUORINE

Fluorine is not considered an element essential for plant or animal life, but has remarkable properties toward preventing dental caries. In fact, fluoride deficiency is probably the foremost nutritional deficiency in the United States (Allaway, 1968).

Soils frequently contain high concentrations of fluorides which occur naturally or may have accumulated over long periods of time through the addition of fertilizers. Because fluorides are for the most part insoluble, little is available for plant uptake; thus, it is extremely doubtful that plants could accumulate concentrations that would be considered detrimental to crops or the animals that consume them.

The effects of fluorides on human and animal nutrition have been reviewed by Underwood (1977). Levels of fluorides toxic to farm animals have been associated with: high fluoride levels occurring naturally in drinking water; the supplementation of fluorides to feed; or aerial deposition of fluoride as a consequence of industrial pollution. Most crops not contaminated by aerial deposition of fluorides contain between 2 and 20 ppm of fluoride (Allaway, 1968), and no cases of fluorosis have ever been ascribed to excessive accumulations of fluorides in plant tissues.

Sewage sludges generally contain low concentrations of fluoride. In a 16 city survey Furr (1976) showed a range of fluorides from 2 to several hundred ppm. Neathery (1976) has suggested that cattle can tolerate 30 ppm of fluoride in their diet without adverse effects. Therefore, there is a potential risk, although small, to grazing livestock if they ingest significant quantities of highly contaminated sludges. Sewage sludges that are not highly contaminated, i.e., less than 100 ppm fluoride, would seem to present little hazard to grazing animals.

LEAD

Lead is believed to be a non-essential element for plant and animal life. The toxic effects of lead on animals have been well documented. Plants, however, do not generally accumulate significant quantities, nor are they very susceptible to lead toxicity. When soluble lead is added to soil it is rapidly precipitated as either phosphates, hydroxides, or carbonates; thus, lead is less available for plant uptake with increasing soil pH and phosphorus levels. Lead added to soils with sludge applications appears to be non-toxic, and often reduces plant uptake of lead when compared to the same soil without sludge additions (see Table 26).

There have been few investigations relating possible lead hazards where sludge is ingested directly by grazing animals. Smith et al., (1976, 1977) fed undigested and anaerobically digested sewage sludge to sheep for a period of 90 days and did not find excessive levels of lead in any of the tissues examined. However, the sludges used in these experiments were relatively low in total lead, i.e., 100 ppm.

It appears from the information available that lead might be hazardous to animals if they were to consume significant quantities of sludge for extended periods of time. However, if sewage sludges that are not highly polluted with lead (less than 1,000 ppm) are used as an agricultural fertilizer and mixed into the soil, there appears to be little chance that grazing animals could consume enough lead to be detrimental to their health.

MERCURY

The mercury content of most municipal sludges is low. Furr's (1976) study of 16 municipal sludges shows a range from 3.6 to 18 ppm (dry weight); however, contents may be high if specific industrial sources are present. Once mixed with the soil, mercury is immobilized by the soil and very little is available for plant uptake; consequently, there is very little increase in plant concentrations as a result of sludge applications.

Mercury ingested and absorbed in the intestinal tract of animals has a long half life; and tends to concentrate in the kidney. However, because of the low concentrations of mercury in sewage sludges and its low availability for plant uptake, there would appear to be little concern that mercury will present a hazard to crops or the food chain as it does in aqueous environments.

NICKEL

Nickel has not been shown to be an essential element for plant life, and various pathological dysfunctions have been shown in chickens, rats and swine where dietary levels were 44 ppb or less (Underwood, 1977). Vanselow (1966) reviewed the nickel content of field grown crops and several species of natural vegetation and found that the average nickel content varied from 0.05

to 5 ppm (dry weight). Vergano (1953) found that concentrations of 60 ppm in oat grain and 28 ppm in oat straw resulted in yield depressions.

Nickel in comparison with other heavy metals such as zinc, copper and cadmium is much more toxic to plants. Apparent nickel toxicity has been reported on acid soils where large quantities of high nickel sludges have been applied. Patterson (1971) applied a sludge containing 6,840 ppm nickel (dry weight) to soils at the rate of 150 mT/ha and found yield depressions at soil pH 5.3, but none at pH 6.8. These findings demonstrate that while nickel can be quite toxic to plants in acid soil, proper soil pH management can prevent toxicity to crops.

Dietary nickel is poorly absorbed by animals and is not cumulative, therefore, it is relatively non-toxic. Even at high intake levels only 1 to 10% of ingested nickel is absorbed, and that which is absorbed is readily excreted in sweat and urine (Underwood, 1977). Growth of chicks has been shown to be depressed when 700 ppm (dry weight) or higher nickel levels were added to the chick's diet. However, nickel toxicity studies with rats showed that diets containing as much as 1,000 ppm nickel (dry weight) did not affect growth rate or reproduction even after 3 to 4 months of continuous feeding (Underwood, 1977).

Sommers (1976) found nickel concentrations in sewage sludges to range from 2 to 3,500 ppm (dry weight) with a mean of 300 to 400 ppm. Nickel can be toxic to plants at high concentrations, but proper soil pH management of sludge treated sites should prevent toxicity to crops. Nickel concentrations needed to produce toxic effects in animals cannot be attained in plant materials. Thus, nickel represents little hazard to crops and virtually none to the food chain.

SELENIUM

Selenium is an essential element for animal life, but as yet has not been shown to be essential for plant life. Limited data pertaining to the selenium content of sewage sludges suggests that selenium is found at only very low concentrations, generally in the 1 to 10 ppm (dry weight) range. Selenium may be deleterious to grazing animals when herbage contains 5 ppm or more. However, the form of selenium in the diet and other dietary factors affect the specific toxicity of selenium (Underwood, 1971).

The CAST report (1976) points out that more needs to be known about the selenium content of sewage sludges and the rate of loss from agricultural soils before potential hazards can be accurately assessed to the use of sewage sludge. However, because of the low concentrations measured in sewage sludges thus far, it is somewhat doubtful that selenium will present a problem as far as sludge utilization on agricultural lands is concerned.

ZINC

Sewage sludges contain fairly high concentrations of zinc ranging from 500 to 50,000 ppm (dry weight) (Chaney, 1973). Excessive applications of sewage sludge containing high concentrations of zinc can lead to zinc toxicity in plants; however, the availability of zinc for plant uptake can be controlled by soil pH management.

Diets consisting of 900 ppm zinc can cause deleterious effects in sheep and cattle (Underwood, 1977); however, concentrations of this magnitude in crops lead to serious plant injury and yield reduction. Therefore, there appears to be a margin of protection from excessive levels of zinc entering the food chain.

An argument can be made for the benefits of increasing dietary zinc. It has been shown that animal and human diets are often deficient in zinc. Hambidge (1974) has reported clinical zinc deficiency symptoms in U.S. teenagers. The application of sewage sludge to agronomic lands that are deficient or marginally sufficient in zinc would not only increase crop yields, but also lead to higher levels of zinc in crops, which would help correct the problem of low dietary zinc in the food chain.

ORGANICS

Information is limited on the amounts of pesticides and persistent organics contained in sewage sludges. The pesticides and refractory organics that represent the greatest concern are those highly chlorinated compounds which resist degradation. These compounds include PCB's (polychlorinated biphenyls), chlordane, DDT (dichlorodiphenyltri-chlorethane), BHC (benzenehexachloride), dieldrin, aldrin, heptachlor, and many of the metabolites from these compounds. The persistence of these chlorinated hydrocarbons is measured in years, while persistence of most other organic pesticides is generally less than a year.

The PCB and pesticide content of a number of municipal sludges is shown in Table 1 (Jelinek and Braude, 1977). Limited amounts of these persistent organics will be added to the soil when sewage sludge is utilized as an agricultural fertilizer. From the information available, sludge additions to soils for fertilizer purposes will not significantly raise the concentration of these chemicals (excluding PCB's) over what is normally found in agricultural soils. Harris (1970) conducted a survey (not related to sludge applications) of insecticide residues in soils of southwestern Ontario, and found DDT residues in almost all soils. Pastures and field crop soils typically contained less than 2 ppm, soils from vegetable crops contained 9.5 ppm, and orchard soils averaged 61.8 ppm DDT. Residues of other cyclodiene insecticides were also detected, but were found in much smaller amounts.

Nash and Woolson (1967) found that the loss of most of these chlorinated hydrocarbons from soils follows first order kinetics. BHC and lindane have a

TABLE 1. PESTICIDES AND PCB CONTENT OF SEWAGE SLUDGES¹

Organic Contaminant	$\mu\text{g/g}$ Dry Weight		Number of Sludges Examined
	Range		
	Minimum	Maximum	
Aldrin ²	N D ⁵	16.2	5
Dieldrin ³	<0.03	2.2	21
Chlordane ²	3.0	32.2	7
DDT + DDE ²	0.1	1.1	7
PCB's ⁴	N D	352.0	83

1. From Jelinek and Braude, 1977.

2. Examined in 1971.

3. Examined in 1971, 1972, 1973.

4. Examined in 1971, 1972, 1973, 1975.

5. Not detected.

half life of approximately 2 years; heptachlor, 2 to 4 years; aldrin, 5 years; dieldrin, 7 years; chlordane, 8 years; and DDT, 10 to 11 years. From the information concerning the half lives of chlorinated hydrocarbons, and assuming that most sludges do not contain concentrations of pesticides higher than those shown in Table 1, it can be concluded that there is little danger that these pesticides will accumulate to hazardous levels when sewage sludge is utilized as a nitrogen fertilizer. In addition, the use of most of these persistent insecticides and PCB's is decreasing; consequently, concentrations of these compounds in sewage sludges will most likely decrease in the future.

Pesticides can be absorbed by plants. However, the amounts of the more persistent chlorinated hydrocarbons that are absorbed from the soils and translocated to aerial portions of the plant are small. An excellent review of plant absorption of pesticides has been published by Nash (1974). Factors which control amounts of specific pesticides that may be absorbed by plants are: the water solubility of the pesticides; the soil and solute concentrations; the size and ionic charge of the pesticide molecule; the soil pH; clay content; soil moisture; soil organic matter content; and the microbial activity of the soil. Many pesticides such as the organophosphates are absorbed rapidly and translocated to the foliage where they control pests; but for the most part, these insecticides have relatively short half lives, and would not be expected to persist in sewage sludges or soils.

The persistent organics that have caused the greatest concern in sewage sludges are PCB's. PCB's are industrial compounds that have been manufactured for the past 50 years and have had a myriad of uses in epoxides,

electrical insulators, and paper. Because of their widespread use and chemical stability, PCB's have become a ubiquitous environmental contaminant and are found in virtually all sludges.

PCB's, like most other chlorinated hydrocarbons, are lipophylic. This fact, combined with their persistence, has led to their biomagnification, particularly in aquatic food chains. Human and animal exposures have occurred from: the epoxides used in food and water containers for livestock; accidents where PCB's have been mixed with animal feeds; fish meat which often have high concentrations of PCB's due to the biomagnification which occurs in aquatic environments. There has been little research examining the fate of PCB's in the soil environment and its subsequent uptake into plant tissues. Fries and Marrow (1978) examined the uptake of three carbon-14 labeled chlorobiphenyls into soy bean plants. They found no detectable levels of residues in any of the plant parts when the chlorobiphenyls were mixed in the surface soil. Significant amounts were lost through vaporization, and absorbed onto lower leaf tissues; thus, the lower leaves often had 10 times the concentration as upper leaves. These results are consistent with those of other researchers (Jacobs et al, 1976; Suzuki et al, 1977) who found none or only extremely low levels of PCB's and PPB's (polybromobiphenyls) translocated from the soil into plant tissues. However, significant concentrations can be found in root crops probably as a result of soil contamination or absorption onto the tuber peel.

When sewage sludge is incorporated into the soil there is little danger that significant levels of persistent chlorinated hydrocarbons will be translocated to aerial portions of crops. But, there still exists a hazard if domestic grazing stock ingest contaminated sewage sludge, as might occur when liquid sludge is applied directly to forages, or when cattle ingest significant quantities of soil highly contaminated with sewage sludge.

Indications are that the high molecular weight compounds (highly halogenated) are absorbed less efficiently into animals than those of lower molecular weights (Fries 1977; Smith et al, 1976). Fries (1977) showed that the maximum steady state milk excretion rate was about 1/2 the daily intake of HCB and DDE, however, this value decreased with increasing halogenation of the compounds or with the ease of metabolism. He estimated that the more persistent compounds were eliminated with half lives of 40 to 70 days. In all cases examined by Fries (1977), the milk fat production was the major factor which determined the rate of elimination of these compounds once they were absorbed into the body. Fries (1977) concluded that guidelines for allowable concentrations of persistent organics in feeds should be no greater than 1/10 the guideline for persistent organics contained in milk fat.

Little work has been done evaluating the uptake and turnover rates of persistent organics in beef cattle or other non-lactating domestic stock. Interpretation of the available research is difficult because the dose and subsequent residues are related to: (a) growth; (b) rate of fat mobilization and deposition; (c) lipid concentrations and interactions; and (d) the relative microsomal activity which metabolizes halogenated hydrocarbons. Estimates of the half life of HCB (which could be assumed to be similar to other

persistent halogenated hydrocarbons) in beef cattle and sheep has been estimated to be between 70 and 128 days (Fries and Marrow, 1977; Henbrey et al., 1975; Avrahami and Steele, 1972). From this information, inferences can be made that the half life of other halogenated hydrocarbons within beef cattle will be of similar length of time.

When sewage sludge is sprayed or applied directly to growing forages or crops, a significant amount of sludge residue may remain adhering to plant tissues. The U.S. Food and Drug Administration is presently taking this viewpoint and is recommending that sludges with PCB's greater than 10 ppm (dry weight) not be applied to lands used for pastures or crop production (EPA, 1977). However, if sludge is incorporated into the soil, significantly higher concentrations of sludge PCB's could be permitted. If sewage sludge is incorporated into the soil to raise agricultural crops, estimates are that as much as 22 kg/ha of PCB's could be applied before the crop would contain more than 0.2 ppm PCB's (Pahren et al., 1977), which is FDA's tolerance level for infant foods and animal feed for food producing animals (Code of Federal Regulations, Title 21).

From the available data, it appears unlikely that sewage sludge could contain concentrations of pesticides or PCB's that would adversely affect crops or the food chain when sewage sludge is incorporated into the soil. However, caution should be exercised when sewage sludge is applied directly to forages which could then be ingested by grazing animals.

EFFECTS OF SLUDGE INGESTION ON LIVESTOCK

Waste products have been recognized as a potential energy source for livestock for a number of years and a great deal of research has been conducted utilizing various animal manure (Anthony, 1974; Ward and Seckler, 1975). Hackler et al., (1975) fed dried activated Chicago sewage sludge (6.46% sludge diet) to sheep and found a nitrogen nutritive value equal to soybean oil or urea-containing rations. Firth and Johnson (1955) fed dried activated Chicago sewage sludge to chicks and baby pigs and found a positive growth response when the sludge was added to chick diets at the rates of 2 and 10%. The growth of baby pigs was depressed when a diet containing 10% sludge was fed; however, there were no ill effects when the sludge diets were reduced to 5% or less.

Research personnel at New Mexico State University have been investigating the nutritive value of sewage sludge to ruminants. Initial rat experiments were designed to evaluate possible adverse or toxic effects of sewage sludge. Rats were fed dried sewage sludge at rates up to 50% of their total diet from weaning to adulthood (Smith et al., 1976; Anonymous, 1979a). Results indicated that the sludge provided no food value to the rats; however, there were no toxicities, no observable teratogenic effects on offspring, or recognizable lesions in internal organs. Subsequent studies utilizing cattle and sheep demonstrated that undigested (raw) sludge had a positive nutritive and nitrogen value that was worth at least half that of cottonseed meal (Smith et al., 1976; Smith et al., 1979). Heifers were fed sewage sludge

(20% of diet) for 68 days in order to evaluate potential adverse effects from the ingestion of high levels of sewage sludge (Smith et al., 1979). The sludge fed heifers gained less weight than controls due to the poor palatability of the sludge diet, and showed slight increases of Fe and Pb in kidney and liver tissues (Table 2). None of the trace metals increased to levels considered abnormal, nor were there any metal increases in muscle tissues (Smith et al., 1979). Additional feeding studies in which cattle consumed a 12% sludge diet for 84 days showed no significant treatment differences in tissue metal concentrations (Table 2) (Smith et al., 1980).

Bertrand et al. (1978) fed sewage sludge (dietary rates of 0, 3.3 and 6.8%) to cattle for 119 days. There appeared to have been slight decreases of Zn and Cu in liver tissues due to the sludge ingestion and slight increases of Pb in kidney tissue (Table 2). No other metals were significantly increased in either kidney, liver or muscle tissues. Bertrand et al. (1980) conducted another cattle feeding study utilizing Chicago sewage sludge which contained much higher concentrations of Cd, Cu, Pb and Fe. There were significant increases of Cd, Pb and Fe in kidney tissues; and Cd, Cu and Fe in liver tissues (Table 2). Trace metal concentrations did not increase in muscle tissues. Average daily weight gains of the cattle were not affected by the sludge ingestion in either of these studies (Bertrand et al., 1978, 1980).

Sludge feeding and grazing studies were conducted by the University of Maryland and the Agricultural Research Service (Anonymous, 1977, 1979). The feeding studies utilized composted sludge which was fed to cows, calves and steers at the rates of 2.3 and 6.3% of the diet (dry weight). The cattle were fed these diets for up to 209 days. Results demonstrated that the compost contained little if any food value. Cadmium, Pb, Cu and Fe increased in either kidney or liver tissues (Table 2); however, there were no significant increases in muscle tissue.

Grazing studies were conducted in 1976, 1977 and 1978 by these same researchers (Anonymous, 1977, 1979). Liquid sludge, compost, or NH_4NO_3 fertilizer was surface applied to pastures. Cattle (cows, calves and steers) were turned out onto the pastures either 1 or 21 days after the sludge applications. Heavy metal analysis of cattle feces grazing the sludge applied pastures indicated that from 2 to 18.6% of the metals contained in feces was from compost or sludge. Cattle grazing pastures 1 day after liquid sludge application showed trace metal levels in feces to be 18.6% of the sludge heavy metals. Assuming approximately 50% digestibility of the forage, the cattle diet would then consist of approximately 9% sludge (dry weight basis). This figure decreased to about 3% (6.1% sludge in feces) when cattle grazed pastures 21 days after sludge application and 1% (2% compost in feces) when compost was applied (Anonymous, 1977, 1979).

During the initial year (1976) of grazing, cattle apparently suffered Fe toxicity from grazing pastures applied with high iron liquid sludge (11% Fe). The following year (1977) sludge containing less Fe (3.7% Fe) was used, and thus eliminated the Fe toxicity problems (Anonymous, 1977). Except for the initial year of the grazing studies, cattle health and performance from grazing pastures after application of liquid sludge was not different from

TABLE 2. HEAVY METAL CONTENT OF KIDNEY AND LIVER TISSUES FROM CATTLE THAT HAVE INGESTED VARIOUS AMOUNTS OF DIFFERENT SEWAGE SLUDGES

Element	% Sludge in diet	Days fed	ug/g (dry weight)		
			Sludge	Kidney	Liver
Source: Smith et al., 1979 †					
Cd	0	-	-	1.19	0.31
Cd	20	68	13	1.49	0.29
Zn	0	-	-	90.8	109
Zn	20	68	950	97.3	84.9
Cu	0	-	-	18.5	146
Cu	20	68	486	16.4	67.2
Pb	0	-	-	2.62 a ‡	1.4 a
Pb	20	68	155	8.43 b	4.23 b
Fe	0	-	-	276 a	148 a
Fe	20	68	8320	422 b	597 b
Source: Smith et al., 1980					
Cd	0	-	-	0.35	0.20
Cd	12	84	135	0.53	0.14
Zn	0	-	-	50	115
Zn	12	84	950	60	95
Cu	0	-	-	139	211
Cu	12	84	486	151	183
Pb	0	-	-	<0.8	<0.8
Pb	12	84	155	<0.8	<0.8
Fe	0	-	-	172	190
Fe	12	84	8320	210	172
Source: Bertrand et al., 1978 †					
Cd	0	-	-	1.1	0.42
Cd	3.3	119	7	1.5	0.60
Cd	6.8	119	7	1.6	0.48
Zn	0	-	-	75.16	132.0 a
Zn	3.3	119	2570	78.64	134.7 a
Zn	6.8	119	2570	76.36	54.0 b
Cu	0	-	-	15.3	38.0 ab
Cu	3.3	119	309	16.1	51.3 b
Cu	6.8	119	309	15.2	25.0 a
Pb	0	-	-	5.60	0.81
Pb	3.3	119	193	7.80	0.78
Pb	6.8	119	193	6.64	1.74

Continued on next page.

Table 2 (Continued)

Element	% Sludge In Diet	Days Fed	ug/g (Dry Weight)		
			Sludge	Kidney	Liver
Source: Bertrand et al., 1980					
Cd	0	-	-	1.2 a	<0.18 a
Cd	6	141	163	55.24 b	10.53 b
Zn	0	-	-	103.0	135.8
Zn	6	141	2501	123.9	117.3
Cu	0	-	-	18.4	52.05 a
Cu	6	141	1365	19.9	122.2 b
Pb	0	-	-	<0.5 a	<0.3
Pb	6	141	774	2.9 b	<0.8
Fe	0	-	-	316.6 a	272.2 a
Fe	6	141	37267	525.0 b	788.0 b

Source: Anonymous, 1979 †

Cd	0	-	-	1.16 a	0.35
Cd	2.3	209	5.58	1.85 b	0.48
Cd	6.3	209	5.58	2.50 c	0.68
Zn	0	-	-	69.3	105
Zn	2.3	209	577	72.6	106
Zn	6.3	209	577	71.6	100
Cu	0	-	-	15.0	37.4 a
Cu	2.3	209	244	16.6	18.4 a
Cu	6.3	209	244	16.3	159.5 b
Pb	0	-	-	2.59 a	1.09 a
Pb	2.3	209	215	6.81 b	2.18 a
Pb	6.3	209	215	2.06 a	6.19 b
Fe	0	-	-	176 a	134 a
Fe	2.3	209	44140	340 b	334 a
Fe	6.3	209	44140	584 c	2728 b

- † Kidney and/or liver concentrations were expressed on a wet weight basis in original source and were converted to dry weights using 33.3% dry matter for liver tissues and 25% dry matter for kidney tissues.
- # Values followed by different letters are significantly different at the 5% level.
- \$ Metal concentration of sludge was not given, but assumed to be same concentrations as earlier study since the sludge source was the same.
- ¶ Only data for steers given here.

cattle grazing non-sludge pastures. Tissue analysis of kidney, liver, muscle, spleen and bone showed no significant increase in heavy metals, other than Fe, for any of the 3 years that the grazing studies were conducted (Anonymous, 1977, 1979).

Fitzgerald (1980) described grazing studies in which cows, calves, and steers grazed pastures in which Chicago sewage sludge (high metal sludge) was applied to pastures by overhead irrigation. Cattle were allowed to graze continuously during the irrigation. Continuous grazing for over six years had not lead to decreased cattle performance or health problems. Analysis of animal tissues showed significant increases of Cd in kidney and liver, and Cu in liver. No data were given for muscle tissues (Fitzgerald, 1980).

These studies have demonstrated that the ingestion of sewage sludge by livestock can result in significant increases of trace metals or persistent organics in cattle tissues; and thus, could be of concern to the human food chain. However, direct health effects to livestock from sewage sludge ingestion appear to be benign. The only exception to this statement was when a high Fe sludge led to apparent Fe toxicity. There appears to be some limited nutritive value from raw or undigested sludge to ruminants whereas, anaerobically digested sludge has been shown to contain little if any food value.

PATHOGENS

Pathogens including enteric viruses, bacteria, protozoan cysts, and helminth ova have been shown to survive anaerobic digestion of sewage sludges, although the numbers of these organisms are usually decreased if not eliminated by this process. Air drying in beds and storage in stockpiles has the potential to completely eliminate these organisms (Burge and Marsh, 1978; Hays, 1977), but the time required needs to be determined.

There is little information regarding virus survival. Poliovirus was found to have survived for at least 28 days when sewage effluent was discharged into a Florida cypress dome (Wellings et al., 1974). Larkin et al. (1976), found that poliovirus 1 survived 96 days when inoculated effluent was irrigated on lettuce and radishes during the winter months. The longest survival during summer months was 36 days. Ward and Ashley (1977) showed that enteric viruses were inactivated after evaporation of water from sludge.

Current EPA regulations for the utilization of sewage sludge state that land cannot be used for the growth of human food crops processed raw in the home or consumed raw until 18 months after sludge application unless the sludge has been treated by a "Process to Further Reduce Pathogens" (i.e. pasteurization) (EPA, 1979).

SLUDGE DISPOSAL AND CHEMICAL COMPOSITION OF METRO DENVER'S SEWAGE SLUDGE

The Metro Denver plant was completed in 1966 and designed to treat 3.71×10^8 liters per day (98 million gallons per day) of wastewater. The residues removed in the process were to be dewatered through dissolved air flotation and vacuum filtration, and ultimately disposed of by flash drying and/or incineration. Early in 1967, it became apparent that the ratio of waste activated sludge to the total sludge mixture was much greater than originally anticipated by the design engineers. The higher quantity of more difficult to dewater waste activated sludge had an adverse effect on the vacuum filter cake solids concentration, which averaged 14% to 18% TS (total solids) instead of the expected 22% to 25%. The adverse sludge ratio also increased the amount of ferric chloride and lime required for vacuum filtration by more than 100%. The increased concentration of chemicals and higher moisture content of sludge led to a serious overload of the flash drying incinerators. Mechanical problems and breakdowns as a result of the overload were exacerbated by ferric chloride induced corrosion failures of the stainless steel components in the flash dryer incinerators.

Operational limitations encountered with the incinerators were odors, as well as particulate and opacity air pollution problems. When the incinerators were initially installed in 1966 they met all existing Colorado air pollution standards. In 1970, these standards were revised drastically to reduce stack emission concentrations for stationary sources. During 1971 the incinerators were operated under a temporary variance while modifications were being made to meet the new standards. It soon became obvious that the incinerators could not be economically modified to comply with the new air pollution standards; thus, because of air pollution, mechanical, and fuel problems, the incinerators were permanently shut down in August of 1971.

In 1969 Metro acquired the use of some land (part of the abandoned Lowry Bombing Range) from the City and County of Denver. This land had been sold to the City by the Federal Government for the purpose of solid waste disposal. Since 1969, a number of sludge disposal or recycling methods have been employed (Baxter et al., 1977). Sludge cake (approximately 15% dry matter) was spread on the soil surface at the rate of approximately 67 mT/hectare, and plowed under the same day. The field was then allowed to lay fallow for about 2 months to eliminate seed germination toxicity. After the 2 months fallow period, a seed bed was prepared and planted to forages such as winter wheat, oats, sorghum, or sudan grass. One or more crops were raised each year to provide forage for grazing cattle and erosion control.

CHEMICAL CONSTITUENTS OF METRO DENVER'S SEWAGE SLUDGE

To explain the differences in trace metal and macronutrient content of Metro's sludges, it will be helpful to describe the physical layout and operation of the treatment plant. The Metro plant was originally designed to provide primary treatment for 1.06×10^8 liters per day, and secondary treatment for 3.71×10^8 liters per day. The reason for the imbalance between

primary and secondary capabilities is that Metro Denver provides secondary treatment for the City of Denver which does not have secondary treatment facilities. The City and County of Denver is one of 22 District members (albeit the largest) and has only primary facilities. Sewage from the City of Denver receives primary treatment at the Denver Northside Sewage Plant. The primary sludge from the Denver Northside Plant is anaerobically digested by City of Denver. Because of the proximity to the Denver Northside Plant, the liquid anaerobically digested primary sludge is pumped to the Metro plant for dewatering and disposal by Metro. Therefore, Metro Denver disposes of two types of sludges; a small quantity of anaerobically digested primary sludge and a large quantity of secondary waste activated + raw primary sludge. Until 1975 the Metro District raw sludges and the anaerobically digested sludge were mixed and conditioned with ferric chloride and lime for vacuum filtration.

Since 1975, these two sludge types have been handled separately. The anaerobically digested sludge has been treated with Hercufloc 90, a poly-electrolyte, to aid in dewatering during vacuum filtration. The sludge cake has then been air dried and either used by the Denver Parks Department on city parks or by Metro in various agricultural studies. The raw primary plus waste activated sludge has been dewatered with the aid of ferric chloride and lime as previously stated, and hauled to the LBR and plowed into the soil surface.

The chemical composition of elements that have been analyzed by Metro on a routine basis are shown in Table 3. Other metals that have been analyzed on a limited basis are shown in Table 4.

Table 5 shows the concentration of refractory organic residues that were found in anaerobically digested sludge, waste activated plus primary sludge, final effluent from the Metro Plant; and primary influent to the Metro Plant. Of 22 organic residues examined (See Table 6) only these 9 were found present. The values shown for refractory organic residues reflect a composite sample that was taken in the spring of 1976.

Analytical methods used for the determination of the various chemical constituents shown here are described in Appendix A.

TABLE 3. CHEMICAL COMPOSITION (DRY WEIGHT BASIS) OF METRO DENVER'S SLUDGE FOR THE YEARS 1975 AND 1976¹

	Anaerobic ²		Waste Activated ³	
	Mean	S.D. ⁴	Mean	S.D.
% TKN	3.9	1.5	4.4	0.9
% P	1.4	0.4	1.6	0.4
% Fe	0.6	0.3	3.2	0.4
% Ca	3.1	0.7	9.7	1.7
Cr ppm	701.	215.	412.	128.
Mn ppm	183.	58.	261.	69.
Ni ppm	222.	87.	190.	57.
Cu ppm	961.	309.	703.	202.
Zn ppm	1443.	297.	1028.	215.
Cd ppm	23.	6.	18.	6.
Pb ppm	1016.	649.	352.	121.

1. Values from the analysis of 90 different sludge samples.
2. Anaerobically digested primary sludge.
3. Mixture of raw primary plus waste activated sludge treated with FeCl_3 and lime.
4. Standard deviation.

TABLE 4. MEAN HEAVY METAL CONTENT OF METRO DENVER'S ANAEROBICALLY DIGESTED PRIMARY SEWAGE SLUDGE¹

Element	µg/g Dry Weight
As	1.3
Hg	11
Mo	40
Se	5
Sn	2.5

1. Means represent from 2 to 5 separate analyses of sludge.

TABLE 5. CONCENTRATION OF REFRACTORY ORGANICS RESIDUES IN SEWAGE SLUDGE, INFLUENT AND EFFLUENT WASTEWATERS

	Primary Influent µg/l	Plant Effluent µg/l	Anaerobically Digested Primary Sludge µg/kg (dry weight)	Waste Activated + Primary Sludge µg/kg (dry weight)
p,p' - DDT	T ¹	T	T	1350
p,p' - DDE	N.F. ²	N.F.	300	630
o,p' - DDT	T	T	N.F.	N.F.
p,p' - DDD	T	T	497.4	435
alpha - BHC	T	0.05	16.1	135
Dieldrin	N.F.	4.6	85.5	235
HCB	N.F.	N.F.	N.F.	33
Chlordane	T	T	1535	4260
AR-1254 (PCB's)	T	T	4252	5030

1. T - Trace concentration below detection limits.

2. N.F. - No residue found.

SECTION 5

EXPERIMENTAL PROCEDURES

SOIL AND PLANT SURVEY OF LOWRY BOMBING RANGE AND CONTROL RANCH

The sludge recycle site or Lowry Bombing Range (LBR) is located in Arapahoe County approximately 42 kilometers southeast of the Metro Denver treatment plant (Figure 1). The vegetation of the site was originally natural pasture land typical of the high plains of eastern Colorado. Arapahoe County has a warm semi-arid climate, rainfall averages 36 centimeters per year, while the average annual temperature is about 10°C.

The dominant soils at the LBR are in the Fondis and Renohill series. The Fondis soils are deep well drained upland soils which have been formed from loessal deposits overlaying the Dawson formation (Pleistocene Age). The Fondis surface soil is free of lime, averages 10 centimeters in depth, silt loam to clay loam texture. The Fondis soils have moderately slow permeability, slow internal drainage, and high available water holding capacity (see Appendix B for soil profile description).

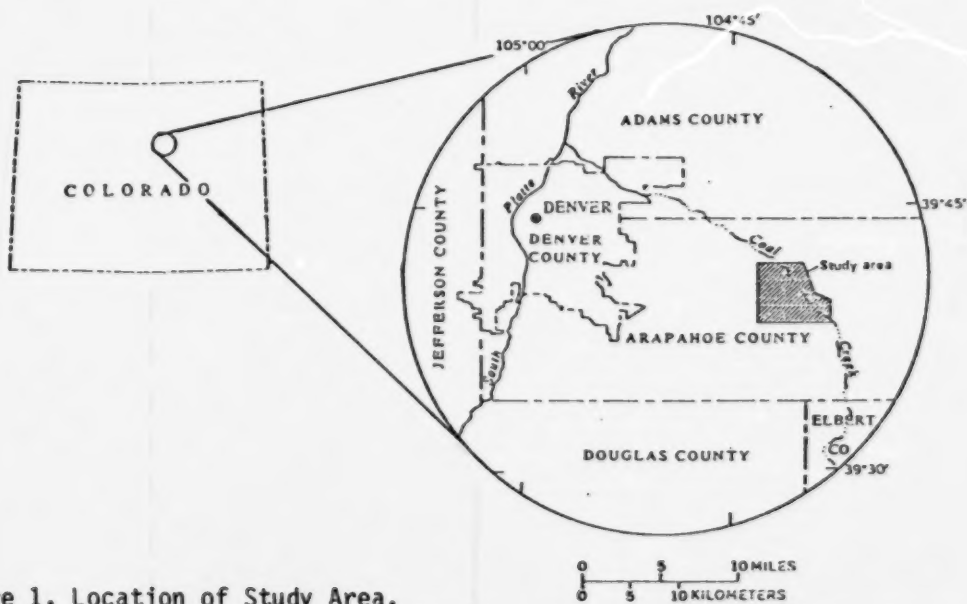


Figure 1. Location of Study Area.

The Renohill series, which has developed on the Dawson formation, is a moderately deep well drained soil. The surface horizon is about 10 centimeters thick, free of lime, and is a dark brown loam. The subsurface soil is approximately 63 centimeters thick, contains free lime, dark brown to dark yellowish brown in color, and ranges from loam to silty clay loam texture. Renohill soils have medium internal drainage, moderately slow to slow permeability, and moderate water holding capacity (see Appendix B soil profile description).

The control ranch is approximately 16 kilometers east of the LBR, with soils predominantly in the Bresser-Truckton series, which are found in association with smaller areas of Renohill and Fondis soils. The Bresser soils have developed on noncalcareous, loessol material. The surface horizon is approximately 13 centimeters thick, free of lime, and dark grayish brown sandy loam. The subsoil is approximately 60 centimeters thick, noncalcareous, brown silt loam to sandy loam. Water infiltration is moderate to rapid with moderate available water holding capacity (see Appendix B for soil profile description).

The Truckton soils are well drained upland soils that have developed on noncalcareous loessol material. The surface layer is typically 15 centimeters thick, dark grayish brown, noncalcareous, loamy sand. The subsoil is approximately 76 centimeters thick, noncalcareous, and grayish brown to brown sandy loam. The Truckton soils have rapid water infiltration and moderate water holding capacity (see Appendix B for soil profile description).

In addition to the Fondis, Renohill, Bresser, and Truckton soil series the control ranch contained some sandy alluvial flood plain soils. These noncalcareous flood plain soils comprised approximately 5% of the total ranch area.

In order to assess the nutrient content and the impact of sludge on the soils at the LBR, soil samples were collected from the perimeter of the site which had never received any sludge applications. Sixteen soil samples were collected from the perimeter; 13 of the soil samples were collected with a soil auger to a depth of 24.5 cm, the other 3 samples were taken from soil pits that were used for soil profile descriptions (see Appendix B for soil profile descriptions). In addition to these samples, 20 fields, which had received a range of sludge applications were also sampled. Six equally spaced soil samples, to a depth of 0.9 meters, were taken from a transect through each field. The control ranch was surveyed by sampling soils to a depth of 24.5 cm in 14 locations from all pastures and soil types represented at the control ranch plus 2 more sites which were from pits that were dug for soil profile descriptions. In addition to the soil samples, forage samples were collected within a 1 meter radius of each soil sampling site. The forage consisted of native pasture from the control ranch and unsludged areas of the LBR. Winter wheat seedlings sampled during the fall was the predominant forage collected from areas that had received sludge applications.

All soil samples were analyzed for: total Zn, Cu, Ni, Cd, Pb, As, Se, Mo, Hg, DTPA extractable Zn, Cu, Ni, Cd, and Pb; pH; NaCHO_3 extractable P; organic matter; NO_3^- ; NH_4^+ ; TKN; conductivity; and selected samples for

C.E.C. (cation exchange capacity). Plant samples were analyzed for Zn, Cu, Ni, Cd, Pb, Hg, As, Se, and Mo. Methods of analyses for soils and plant tissues are described in Appendix A. A number of soil and plant samples were also analyzed for refractory organics. The organic compounds examined in this study (Table 6) were chosen because these compounds represent a potential hazard to the human population. These pesticides are presently being examined by the U.S. Environmental Protection Agency in their National Human Monitoring Program for Pesticides. Methods of analysis and detection limits for the various sample types are shown in Appendix A.

HEALTH AND TRACE ELEMENT LEVELS IN BEEF TISSUES FROM CATTLE GRAZING SLUDGE AMENDED SOILS AT THE LOWRY BOMBING RANGE

The Metro Denver Sewage Disposal District No. 1 has applied sludge since 1969 to a 810 hectare site called the Lowry Bombing Range (LBR). Sludge filter cake has been applied to the soil surface at the rate of approximately 67 dry mT/ha/year, plowed under and after a two-month fallow period, planted with a forage such as winter wheat, oats, sorghum, or sudan grass. A herd of 300 to 500 beef cattle have continuously grazed the entire area; thus, these cattle have had ample opportunity to ingest sludge contaminants.

TABLE 6. LIST OF ORGANIC RESIDUES THAT WERE EXAMINED DURING ANALYSIS¹.

PCB's (AR-1254) (Polychlorinated biphenyl)	thiodan
p,p' - DDT (Dichlorodiphenyltrichloroethane)	chlordane
o,p - DDT	oxychlordane
p,p' - DDE (Dichlorodiphenyldichloroethylene)	heptachlor epoxide
o,p - DDE	Hexachlorobenzene
p,p' - DDD (Dichlorodiphenyldichloroethane)	aldrin
alpha-BHC (Benzene hexachloride)	dieldrin
beta -BHC	methoxychlor
lindane	mirex
heptachlor	TNC (Transnonachlor)
endrin	dacthal

1. Malathion was also analyzed in a few plant and animals tissue and was not detected.

A number of cattle feces from the LBR were randomly collected and analyzed for Ti in order to estimate the quantity of soil consumed by cattle from grazing the LBR (Healy, 1968). Feces samples were collected from the ground surface as chips. To minimize possible soil and dust contamination only the interior of the chips were taken for analysis.

During the fall of 1975 cows from the LBR were compared with another herd that grazed similar land located approximately 16 kilometers east of the LBR but had never had sewage sludge applied to it. A group of 12 older cows from the LBR that had been exposed to sludge since the inception of sludge incorporation (1969) were slaughtered and compared with 6 control cows taken from the control ranch. Both groups of animals were antemortemly inspected by a veterinarian and all organs were examined postmortemly by a pathologist.

The animals were killed with a captive-bolt pistol, and bled by severing jugular blood vessels. Tissues taken for analysis were removed using stainless steel knives. Care was taken to avoid contamination with gut or foreign material. Whole kidneys were taken for analysis and slices of other tissues were taken transversely through each organ to obtain a composite sample of the whole organ. Bone plus bone marrow samples were taken from the proximal half of the tarsal bone, and muscle (*ulnaris lateralis*) samples were taken from the same forearm. All samples were stored frozen in acid washed plastic containers. These tissues were later analyzed for As, Cd, Cu, Hg, Mo, Ni, Pb, Se, and Zn. In addition, bone samples were analyzed for F. Identical tissue samples were stored frozen in acid washed glass containers for later refractory organics analysis. The specific residues that were analyzed for are shown in Table 56. Methods of metal analysis and refractory organics analysis are described in Appendix A. Comparison of treatment means was evaluated statistically using Student's t-test.

INVESTIGATION OF HEAVY METAL CONTENT IN KIDNEY AND LIVER TISSUES FROM CATTLE OF KNOWN AGE AND NO PREVIOUS EXPOSURE TO ELEVATED LEVELS OF HEAVY METALS

Veterinarian examination of cattle from the LBR and the control ranch at the time of slaughter revealed that the cattle from the LBR were older than the cattle from the control ranch. The differences in age between the two groups of cattle could not be quantified, and thus added an unexpected variance to the study. Heavy metal analysis of kidney and liver tissues revealed significant differences between the two groups, which then could not be definitely related to sludge effects or age effects. Therefore, another study was conducted in an attempt to quantify any changes in heavy metal content of cattle tissues that might occur with age.

Cattle were selected on the criteria that exact birthdates were known, and that they had no previous exposure to sewage sludge or elevated levels of heavy metals. One group of 25 Hereford range cows were chosen because they had been raised from birth on the same ranch from eastern Colorado. Fourteen animals included in the study were dairy cows from the CSU dairy farm. Four other animals were range cows from the University of Wyoming. Twelve young steers (18 months old) which were used as controls for feeding trials at C.S.U. were also included. Concentrations of Cd, Cu, Fe, Zn, and Pb were determined in kidney and liver tissues as a function of age. Specific

TABLE 7. INGREDIENTS OF PELLETTED BASAL CONCENTRATE MIX (BCM)

Ingredient	%
Corn	87.0
Cottonseed Meal	7.2
Molasses	5.0
Limestone	0.7
Salt	0.1
Vitamin A ¹	- -

1. Vitamin palmitate to supply 40,000 IU/animal/day.

methods of analysis are described in Appendix A.

INGESTION OF METRO DENVER SEWAGE SLUDGE BY BEEF CATTLE

Metro Denver anaerobically digested primary sludge cake was placed on plastic sheets and air dried to a 35.3% moisture content. The sludge was then ground through a hammer mill and mixed with a basal concentrate mix (BMC) (Table 7) in a horizontal mixer. Three different diet mixtures were pelleted, which consisted of a basal mixture for control steers, and two sludge mixtures for the sludge fed steers (Table 7). The BCM plus sludge mixtures were made up to approximate a final sludge diet of 0, 4, and 12% sludge. Steers were started on 0.45 kg of pellets per head per day plus silage and hay ad lib. During a one month period the diet was gradually changed to 1.81 kg of silage per animal per day and pellets were fed ad lib. The amount of diet components actually consumed by the treatment cattle during the experiment is shown in Table 8. No effort was made to disinfect the sludge, and analysis of pathogens in the sludge was not made.

Table 9 shows the ash, dry matter, protein, and fiber content of the pellets containing sludge fed to the cattle. The ash and fiber content of the diets increased with increasing sludge additions, while the crude protein content remained unchanged.

The trace element composition of all diet components is shown in Table 10. The trace element compositions of the diets consumed by the cattle (Table 11) were calculated by summing the amount of trace metals contained in each diet component that was actually consumed by each treatment group (Table 8) and dividing by the total dry weight of those diet components.

TABLE 8. AMOUNT OF DIET COMPONENTS CONSUMED BY TREATMENT CATTLE DURING THE METRO DENVER SLUDGE FEEDING TRIAL

	Kg (Dry Weight)		
	0% Sludge Diet	4% Sludge Diet	12% Sludge Diet
Basal Concentrate Mix	4056	4045	3497
Sewage Sludge	0	198	587
Silage	1042	1059	1031
Hay	99	102	102
Actual Sludge Consumption (%)	0	3.7	11.3

TABLE 9. PERCENT ASH, DRY MATTER, PROTEIN, AND FIBER CONTENT OF PELLETS FED TO TREATMENT CATTLE

Pellets	% Dry Weight	% Ash	% Crude Protein	% Fiber ¹
0% Sludge	89	2.9	14	4.5
4% Sludge	91	7.8	15	8.4
12% Sludge	90	13.9	14	13.2

1. % acid detergent fiber.

TABLE 10. MEAN TRACE ELEMENT COMPOSITION OF METRO DENVER SEWAGE SLUDGE AND DIET COMPONENTS ADDED TO THE DIETS OF CATTLE¹

Element	Sludge	$\mu\text{g/g}$ (Dry Weight)		
		BCM	Silage	Hay
As	1.31	0.03	0.04	0.03
Cd	21	0.03	0.014	0.03
Cu	710	3.1	3.7	3.2
Fe	12,800	140.	145.	N D ²
Hg	11	<0.005	<0.005	<0.005
Mo	40	0.31	0.23	0.30
Ni	125	0.6	0.6	0.6
Pb	780	0.13	2.1	0.10
Se	5.4	0.16	0.15	0.16
Zn	1500	27	16	26
F	200	10	11	N D
Ca	44,000	3000	2500	N D
Mg	2980	1520	1280	N D
P	16,000	3300	2000	N D

1. Mean of two composite samples taken before mixing into basal diet concentrate mix.
2. Not determined.

The cattle used in this study were approximately 15 months old and were part of a larger lot of black white faces, Angus, and Angus Charlois crosses which were purchased by CSU for research work. The animals were selected for uniformity of weight and divided into three treatment groups of six animals each. The feeding trials began on August 29, 1975, and were terminated 94 days later on December 1, 1975, after the cattle had reached a finishing weight of about 450 kg.

The cattle were slaughtered at the end of the 94 day feeding study. The animals were killed with a captive-bolt pistol, and bled by severing jugular blood vessels. Tissues taken for analysis were removed using stainless steel knives. Care was taken to avoid contamination with gut or foreign material. Whole kidneys were taken for analysis and slices of other tissues were taken

TABLE 11. TRACE ELEMENT COMPOSITION OF DIETS AMENDED WITH METRO DENVER SEWAGE SLUDGE¹

Element	% Sludge in Diet		
	0	4	12
	$\mu\text{g/g}$ (Dry Weight)		
As	0.03	.08	0.18
Cd	0.03	0.79	2.4
Cu	3.2	29.	89.
Fe	138.	600.	1560.
Hg	<0.005	0.4	1.2
Mo	0.29	1.7	3.1
Ni	0.6	5.	16.
Pb	0.5	29	80.
Se	0.16	0.35	0.75
Zn	25.	79.	190.
F	2.	9.	22.
Ca	2800	4400	7500
Mg	1400	1500	1600
P	3000	3400	4400

1. Mean of two separate composite samples of diet components.

transversely through each organ to obtain a composite sample of the whole organ. Bone plus bone marrow samples were taken from the proximal half of the tarsal bone, and muscle (ulnaris lateralis) samples were taken from the same forearm. Specific tissues sampled were kidney, liver, bone, muscle, blood, fat, lung, brain, and spleen. All animals were antemortem inspected by a veterinarian and examined postmortem by a pathologist. The methods of analysis used for the tissue samples are described in Appendix A.

In addition to heavy metals, samples of fat, kidney, brain, blood, liver and muscle tissues were analyzed for the 22 refractory organics listed in Table 6. Concentrations of the various refractory organics detected in the sludge, silage, feed or water is shown in Table 12. Methods used in the determination of the refractory organics and their detection limits in the various tissue types is discussed in Appendix A.

TABLE 12. CONCENTRATION AND RANGE OF RESIDUES IN (ppb WET WEIGHT) SLUDGE, SILAGE, FEED, AND WATER USED IN METRO DENVER SLUDGE FEEDING TRAIL

Compound	Sludge ²	Feed ¹		Silage ³	Water
		0% Sludge	12% Sludge		
HCB	ND - <4.0	ND ⁴	< 4.0	ND	ND
p,p' -DDE	30 - 70	ND	<10 - 13	ND	<0.09
p,p' -DDD	ND - 30	ND	ND	ND	ND
Dieldrin	80 - 140	ND	ND	ND	ND
Chlordane	1160 - 1600	ND	160 - 170	ND	ND
Oxychlordane	80 - 116	ND	<1.0	ND	ND
AR-1254	1250 - 2850	ND	<500.	<40.0	ND

1. Percent moisture of feed 10%.
2. Percent of moisture of sludge 30%.
3. Percent moisture of silage 67%.
4. ND - Not detected.

INGESTION OF FT. COLLINS SLUDGE BY BEEF CATTLE-3 MONTH INGESTION PERIOD

A second feeding trial was conducted using Ft. Collins anaerobically digested sludge which had a different trace metals content (Table 15). The principal difference in metals between Ft. Collins and the Metro Denver sludge was that the Ft. Collins sludge contained approximately 100 ppm Cd and 1700 ppm Cu (dry weight basis) compared to 20 ppm Cd and 700 ppm Cu (dry weight basis) in the Metro Denver sludge.

The Ft. Collins sludge was anaerobically digested primary plus waste activated sludge which had dried in earthen drying beds for approximately one year. The surface layer or top 4 cm of dried sludge was removed from the basin and ground through a hammermill. The sludge was then mixed in a horizontal mixer, and stored in burlap sacks for future use in the diets. Prior to mixing sludge in the diets, the sludge was pasteurized by heating it, in the sacks, to 90°C internal temperature and holding it at that temperature for 12-24 hours.

Two different pelleted mixtures were made, consisting of a BCM (Table 7) for control steers, and a sludge containing mixture for the sludge fed steers. The pelleted diet mixtures were fed ad lib. with a constant amount of roughage (1.81 kg of silage and hay per head per day). The amounts of individual diet components that were consumed by the cattle during the experiment are shown in Table 13. Table 14 shows the ash, dry matter, crude protein, and fiber content of the pellets fed to the cattle. The ash, fiber

TABLE 13. AMOUNT OF DIET COMPONENTS CONSUMED BY CATTLE DURING THE FT. COLLINS SLUDGE FEEDING TRIAL

	kg (Dry Weight)	
	0% Sludge Diet	12% Sludge Diet
Basal Concentrate Mix	4240	3879
Sewage Sludge	0	652
Silage	1084	1095
Hay	68	68
Actual Sludge Consumption (%)	0	11.5

and crude protein content of the diets increased with sludge additions. While the crude protein content (based on total Kjeldahl nitrogen) increased with sludge addition, this protein was most likely undigestible protein and did not increase the food value of the diet.

The cattle used in this study were similar to cattle used in the first Metro Denver sludge feeding trial. They were approximately 15 months old and were part of a larger lot. The animals were selected for uniformity of breed (Herefords), weight, and divided into two treatment groups of six animals each. The feeding trials began on November 12, 1976, and were terminated 106 days later on February 23, 1977, after the cattle had reached a finishing weight of about 465 kg.

The cattle were slaughtered at the end of the 106 day feeding study. Sampling procedures and tissues analyzed were the same as those previously described for the Metro Denver sludge feeding trials. As in the first feeding trial, the cattle were antemortemly inspected by a veterinarian and post-mortemly examined by a pathologist.

Specific tissues taken for heavy metal analyses were kidney, liver, bone, muscle, lung, brain, and spleen. All tissues were analyzed for Zn, Cd,

TABLE 14. PERCENT ASH, CRUDE PROTEIN, ACID DETERGENT FIBER, AND ASH-FREE ACID DETERGENT FIBER OF PELLETS

Pellets	Crude Protein	ADF	%	
			Ash-Free ADF	Ash
Control	11.87	5.98	5.88	2.78
Ft. Collins Sludge Pellets (12%)	14.83	11.48	8.04	8.93

Cu, Hg, and Pb. Methods of analysis are discussed in Appendix A.

SHOULD
HEAD
BEGIN
SECTION
HERE

Table 15 shows the heavy metals concentrations of the Ft. Collins sludge that was added to the cattle diets. As can be seen there was little difference between the Zn concentrations of the two sludges. However, the Cd concentration was 5 times the Cd concentration of the Metro Denver sludge, with a Cd:Zn ratio of approximately 5.6% as compared to a Cd:Zn ratio of approximately 1.4% in the Metro Denver sludge.

The Cu concentration of the Ft. Collins sludge was 1700 ppm which was considerably higher than the Metro District sludge which had a Cu concentration of 710 ppm.

Shown in Table 16 are the concentrations of heavy metals contained in the diets fed to the cattle. Dietary concentrations were calculated in the same manner as in the Metro Denver sludge feeding trial.

All tissues and diet components were also analyzed for the same refractory organics as examined in previous studies (see Table 6). The concentration of organic residue found in the Ft. Collins sludge, feed, and silage are shown in Table 17. The concentration of the various organic compounds are similar to the levels found in the first feeding trial utilizing Metro Denver sewage sludge (see Table 12). The Ft. Collins sludge contained slightly higher concentrations of p,p'-DDE and AR-1254, while the

TABLE 15. MEAN CONCENTRATION OF HEAVY METALS CONTAINED IN METRO DENVER SLUDGE, FT. COLLINS SLUDGE AND DIET COMPONENTS OF FT. COLLINS FEEDING TRIALS

Element	µg/g (Dry Weight)			
	Metro Denver	Ft. Collins ¹	BCM ¹	Silage ¹
Zn	1500	1700	27	24
Cd	21	98	0.11	0.27
Cu	710	1700	5.7	7.9
Fe	12,800	10,000	130	145
Hg	11	18	<0.01	<0.02
Pb	780	470	0.67	1.6
Ca	44,000	29,000	3000	2500
Mg	2980	3900	1500	1300
P	16,000	19,000	2300	2000

1. Mean of 6 separate composite samples.

SHOULD
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

TABLE 16. CONCENTRATION OF Zn, Cd, Cu, Hg, AND Pb OF DIETS AMENDED WITH FT. COLLINS SLUDGE

Element	$\mu\text{g/g}$ (Dry Weight)		
	Ft. Collins Dietary Sludge		Metro Denver
	0%	12%	12%
Zn	26.3	236.	190.
Cd	0.14	11.3	2.4
Cu	7.7	213.	89.
Fe	130.	1300.	1560.
Hg	<0.02	2.6	1.2
Pb	0.86	56.6	80.
Ca	2400.	5800.	7500.
Mg	1500.	1730.	1600.
P	3000.	4800.	4400.

TABLE 17. MEAN CONCENTRATION OF REFRACTORY ORGANICS DETERMINED IN FT. COLLINS SLUDGE FEED AND SILAGE¹ (ppb, WET WEIGHT BASIS)

Compound	Sludge ²	Silage ³	Feed ⁴	
			0% Sludge	12% Sludge
p,p'-DDE	121	<2.0	<10.0	31
alpha-BHC	<2.5	<1.0	<10.0	<10.0
beta-BHC	<12.5	ND ⁵	ND	<10.0
Dieldrin	39	ND	<10.0	<10.0
Oxychlordan	28	ND	ND	<10.0
HCB	<5.7	<1.0	ND	<10.0
Chlordane	1047	ND	ND	188
AR-1254	4434	<40.0	ND	758

1. Mean of 6 separate samples.
2. Ca. 5% moisture.
3. Ca. 67% moisture.

4. Ca. 10% moisture.
5. ND - Not detected.

Denver sludge contained slightly higher levels of oxychlordane, and p,p'-DDD. For the most part, however, the two sludges were comparable, as were the sludge feed mixtures that were fed to the cattle during the two experiments. Trace concentrations of DDE, alpha-BHC, HCB, and AR-1254 were found in the silage, which differed from the first feeding trial, where AR-1254 was the only organic residue detected.

The methods of analysis for the various sample types and their detection limits are shown in Appendix A.

INGESTION OF FT. COLLINS SLUDGE BY BEEF CATTLE: 9 MONTH INGESTION PERIOD

A third sludge feeding trial was conducted using Ft. Collins anaerobically digested sludge. The sewage sludge was acquired from the same sludge drying basin as previously described. Sixteen Hereford cows were purchased from the C.S.U. Akron Experiment Station. The cows ranged in age from 4 to 7 years. Sixteen 6 month old Hereford steers were purchased from a local source. Prior to purchase all steers had been vaccinated for infectious bovine rhinotracheitis, leptospirosis, and black leg. All animals at the time of purchase were dipped in Copper-tox solution to control external parasites and fed Thiabendazole for two days to control internal parasites. Animals were also injected with 1×10^6 I.U. vitamin A, 1×10^5 I.U. vitamin D, and 100 I.U. vitamin E at the time of purchase and repeated at the time of each biopsy.

The cows were lotted into two uniform groups of 8, on the basis of weight and age, so that each group had an average age of 5. Steers were lotted into two groups of 8 according to weight. One group of cows and steers were placed on a control diet and one group of cows and steers were fed a diet containing approximately 12% (dry weight) Ft. Collins anaerobically digested sludge for a period of 9 months. The cow diets were fed at approximately maintenance levels, while the steer diet was fed and adjusted to maintain a 0.8 kg per head per day weight gain.

The sewage sludge as delivered to the C.S.U. farm was approximately 78% dry matter. Because of the large quantity of sludge that had to be handled, the sludge was not dried prior to being fed to the cattle. Batches of sludge were put through a hammer mill, mixed in a horizontal mixer and stored in burlap sacks. The moisture content of the mixed sludge was determined and the amount fed adjusted to achieve a 12% dry weight sludge diet. Silage, alfalfa, and protein supplement were weighed and added to the feed bunkers of the treatment cattle, sludge was then added to the top of the feed in the bunker and mixed into the feed. Cattle receiving the sludge diet readily accepted the added sludge and did not attempt to separate out feed components as it was consumed.

At the conclusion of the 9 month sludge feeding period, 4 animals from each group were slaughtered, and samples of kidney, liver, muscle, and fat tissues taken for analysis. The remaining animals were kept on test for another 4 months, but sludge was removed from the diet. The 16 remaining

animals were then slaughtered and tissue samples taken at the end of this 4 month "withdrawal" period.

Biopsies were taken in order to assess the rate of retention of heavy metals into tissues. Liver and bone biopsies were taken from all animals 0, 90 and 180 days after sludge was added to the diet. Half the animals were slaughtered on day 279, the remaining half had liver and bone biopsies taken. Further biopsies were taken from the remaining cattle 42 and 84 days after the beginning of the "withdrawal" period.

Biopsies were taken while animals were restrained in a chute. The surgical site for the liver biopsies was on the animals right side in the last intercoastal space and approximately 8 cm ventral to the loin muscle. The surgical site was shaved of hair, scrubbed with Betadine surgical scrub, and sprayed with Betadine solution. A 2.5 cm incision was made into the peritoneum. A 0.5 to 0.7 cm trocar and cannula was inserted through the incision to the liver, the trocar was then withdrawn as the serrated cannula barrel was rotated and inserted into the liver. A vacuum was applied to the cannula and the liver sample was then aspirated through a connecting vacuum tube and deposited in a clean collection flask. The biopsy was then placed in an acid washed plastic vial and stored frozen until analysis.

Bone biopsies were taken from the tail. The tail was shaved and surgically prepped. A local anesthesia (5-7cc of 2% Lidocaine) was injected into the spinal column between coccygeal vertebrae 1 and 2. The tip of the tail was opened and an entire vertebra removed. The vertebra was stored in an acid washed plastic vial and stored frozen until analysis. The wound was closed using wound clips and then sprayed with Betadine.

After slaughter, tissue samples of kidney, liver, bone, fat, and muscle were collected as previously described for other experimental animals. Kidney, liver, and muscle samples were homogenized in a blender, aliquots were oven dried, digested, and analyzed as described in Appendix A. Bone samples were analyzed using the same procedures, but the bone was not homogenized. The whole vertebra was digested in the case of bone biopsies, while only a small section (1.5cm length) of the tarsal bone was used. No attempt was made to remove bone marrow. Tissue samples of bone, muscle, kidney, and liver were analyzed for Fe, Cu, Ni, Cd, and Pb.

Omental fat samples were collected as previously described, and analyzed by the procedures outlined in Appendix A.

At the time of slaughter the animals were antemortem and postmortemly inspected by a veterinary pathologist. Tissues for histopathological examination were taken by the pathologist.

PATHOGEN SURVIVAL DURING AIR DRYING OF DIGESTED SLUDGES

There are four groups of enteric pathogens in sewage wastes; viruses, bacteria, protozoan cysts, and helminthic ova. Anaerobic digestion has been

shown to eliminate protozoan cysts (Hays, 1977). As discussed in several reviews (Burge and Marsh, 1978; Hays, 1977), the other three groups of organisms are significantly reduced in numbers, but at times have been found to survive this process.

When sludge is applied to land, the pathogens present may be a potential source of disease to field workers, people of nearby communities and to people utilizing the animal or plant products from the sludge treated field. Air drying in outdoor earthen beds before utilization of the sludge has the potential of reducing or eliminating the pathogen populations remaining in anaerobically digested sludge to innocuous levels. Reductions occur through natural die-off; predation; antagonism and competition from other organisms; through dessication; or other means that have not been completely defined. However, the sludge residence time in drying beds for adequate pathogen destruction needs to be defined.

Since the amount of resources prohibited the study of the survival of all pathogens, a few pathogens and indicator organisms were selected. Salmonellae bacteria and ascaris ova were chosen because they are important pathogenic organisms. Salmonellae are especially important because regrowth in sludge under the right conditions is possible. Fecal coliforms were used because these are traditional indicators for fecal contamination of water, and comparison of their survival with that of salmonellae and the other organisms could aid in determining whether they will be useful as an indicator of pathogen survival as influenced by the air drying of sludge. Bacteriophage f2 was used because under most conditions it is more persistent than other viruses. In trenching studies with digested sludge it has exhibited a half life of 6.6 to 7.2 days (Cramer and Burge, Unpublished Data) or in terms of the time for a 10-fold reduction, 22 to 24 days. For comparison, Larkin et al., (1976), found poliovirus in fecal material applied to vegetables required only 5 to 6 days for a 10-fold loss. Further, like enteroviruses and unlike other phages it cannot replicate in the sludge, because its hosts, the Hfr strains of Escherichia coli, are extremely rare.

Three studies were conducted to span the different seasons. The studies were initiated on January 13, 1977 (Winter), April 20, 1977 (Spring), and July, 1977 (Summer-Fall). A fourth was initiated March 29, 1978. In the first three studies the concentrations of ascaris ova were either too low for detection or so low that inactivation measurements could not be made. The fourth study was initiated so that ascaris ova could be seeded into the sludge at high enough concentrations so that its fate could be determined. In all four studies f2 bacteriophage was seeded into the sludge to follow its survival. In the fourth study T7 bacteriophage was seeded along with f2.

Both bacteriophages were grown in the U.S.D.A. Biological Waste Management and Soil Nitrogen Laboratory, Beltsville, Maryland and mixed at Denver, Colorado with the liquid sludge as it filled a 23,000 liter tank truck. The liquid sludge was then transported to the Northern Colorado Research and Demonstration Center near Greeley, Colorado and pumped into earthen basins (7.6 m x 7.6 m x 0.8 m deep) to a depth of about 0.6 m. The beds contained ceramic candles placed at a depth of 91 cm below the bed bottoms to sample the sludge leachate. The sludge was sampled at three week intervals until

BEGIN
FIRST
LINE OF
TEXT
HERE

Typing Guide Sheet

CENTER
OF PAGE

TOP OF
IMAGE
AREA

the microbial tests were essentially negative. When present, leachate samples were taken concurrently with the sludge samples. The sludge was analyzed for seeded bacteriophages, salmonellae, total coliforms, fecal coliforms, ascaris ova, and water content. The leachate was analyzed for the bacteriophages, salmonellae, total coliforms, fecal coliforms and chloride.

In the fourth study, ova of Ascaris suus were obtained from adult nematodes collected from a slaughter house and seeded into the sludge at the drying bed site. Three fifty gallon drums with both ends cut out were cut in half and set up in the sludge after the basin had been filled. To prevent dilution, the ova were seeded into the sludge which was enclosed by the drums.

Methods used for the assay of ascaris ova, salmonellae, coliforms, f2 and T7 bacteriophages, and the growth of f2 and T7 bacteriophages are given in Appendix A.

REPLY
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

SECTION 6

RESULTS AND DISCUSSION

SOIL AND PLANT SURVEY OF LBR AND CONTROL RANCH

Heavy Metals and Nutrients in Soils and Forages at the LBR Control Ranch

A comparison of selected chemical constituents of soils from the perimeter of the LBR (areas that had never received sludge) and control ranch are shown in Table 18. The pH and C.E.C. of the control ranch were substantially lower than soils from the LBR, while the other chemical constituents shown are similar.

TABLE 18. SELECTED CHEMICAL CONSTITUENTS OF SOIL FROM THE CONTROL RANCH AND SOILS FROM THE LBR WHICH HAD NEVER RECEIVED SLUDGE APPLICATIONS

	LBR Control		Control Ranch	
	Mean	Range	Mean	Range
pH	7.0	6.6 - 7.5	6.4	5.9 - 6.7
C.E.C meq/100 g	28	20 - 34	9.1	5 - 14
P (ppm)	8	1 - 19	19	8 - 50
NO ₃ ⁻ -N (ppm)*	<2.4	<1 - 6	<3	<1 - 8
NH ₄ ⁺ - N (ppm)*	3.3	2 - 4	4	2 - 8
TKN (ppm)*	1145	730 - 1530	800	370 - 1900
Organic Matter (%)	1.0	0.6 - 1.5	1.0	0.6 - 1.8
Conductivity (mmhos/cm)	0.20	0.11 - 0.33	0.16	0.06 - 0.24

* NO₃⁻, NH₄⁺, and TKN expressed as ppm N.

TABLE 19. MEAN AND RANGE OF TOTAL HEAVY METAL CONTENT OF SURFACE SOILS FROM THE CONTROL RANCH AND LBR WHICH HAD NEVER RECEIVED SLUDGE APPLICATIONS

Element	µg/g (Dry Weight)			
	LBR Control		Control Ranch	
	Mean	Range	Mean	Range
Zn	50.8	39.5 - 62.5	26.5	6 - 44.5
Cd	0.43	0.18 - 0.72	0.28	0.12 - 0.52
Cu	10.6	6.75 - 18.8	3.82	1.75 - 5.75
Ni	11.8	8.25 - 16.0	4.42	1.25 - 6.25
Pb	18.2	14.0 - 22.0	13.2	3.50 - 24.0

Table 19 shows that the total metal concentrations of Zn, Cd, Cu, Ni, and Pb were generally higher at the LBR than at the control ranch. Other metals, As, Se, Mo, Hg, and Sn, averaged <3 ppm, <1 ppm, <0.9 ppm, 25 ppb, and 5.5 ppm respectively, with only slight differences between the two sites.

Although the LBR soils had a higher native metal content than the control ranch, this is not reflected in the heavy metal content of forage tissues that were collected at each soil sampling site. As shown in Table 20, there were no significant differences in metal concentrations. Mean concentrations of As, Se, Mo, and Hg were 0.25 ppm, 0.35 ppm, 1.65 ppm, 24 ppb, respectively, for both sites. The variation between sites was insignificant.

TABLE 20. MEAN AND RANGE OF HEAVY METALS OF FORAGES TISSUES FROM UNSLUDGED AREAS WITHIN THE LBR AND CONTROL RANCH

Element	µg/g (Dry Weight)			
	LBR Control		Control Ranch	
	Mean	Range	Mean	Range
Zn	11.7	7.00 - 23.7	13.7	7.25 - 24.25
Cd	0.11	0.02 - 0.18	0.3	0.12 - 0.38
Cu	2.28	1.25 - 4.50	2.17	1.38 - 2.75
Ni	<0.6	<0.25 - 1.25	0.78	0.25 - 2.75
Pb	<2.3	<0.65 - 5.12	2.98	2.00 - 9.75

The fact that there was little difference in the heavy metal content of forage tissues between the two sites before the application of sludge indicates that the cattle grazing these lands were most likely exposed to similar amounts of heavy metals before the application of sewage sludge to the LBR.

Because there has been a broad range of sludge applications to the LBR it is difficult to define the exact heavy metal and nutrient status of soils and plants for the site as a whole. Table 21 shows the mean concentration of heavy metals in surface soil samples from the LBR where no sludge has been applied and the median and range of surface soil samples taken from the 20 sludged fields that were surveyed. The median is employed in describing the sludged areas because it is a more realistic description of central tendencies of the heavy metal status of the soils. The concentration of molybdenum was measured in one field which had received the highest sludge application (more than 420 tons/hectacre) and was found to range between 1.25 ppm and 1.90 ppm with a mean of 1.56 ppm. Since the concentrations were low in even a highly sludged field, the rest of the areas receiving sludge were not analyzed for total soil molybdenum. Table 21 shows that the addition of sludge to the LBR significantly raised the concentrations of most heavy metals, however, concentrations of As, Se, and Hg which were found in low concentrations in the sewage sludge (see Table 4) were not appreciably increased in the soil by sludge additions

TABLE 21. MEAN, MEDIAN AND RANGE OF HEAVY METALS IN SURFACE SOIL SAMPLES FROM AREAS OF SLUDGE APPLICATION AND CONTROL AREAS WITHIN LBR

Element	$\mu\text{g/g}$ (Dry Weight)				
	No Sludge Applications		Areas of Sludge Application		
	Mean	Range	Median	Range	
Zn	50.8	39.5 - 62.5	93	40	-252
Cd	0.43	0.18 - 0.72	1.45	0.43	- 4.40
Cu	10.6	6.75 - 18.8	27.2	6.0	- 82.5
Ni	11.8	6.0 - 43	21.8	6.00	- 43.0
Pb	18.2	14.0 - 22.0	36.2	14.2	-101
As	<3	<3 - 3	3	<3	- 5
Se	<1	<1	1	<1	- 2
Hg	<0.027	0.010 - 0.090	0.150	<0.010	- 0.580

A comparison of unsludged areas versus sludged areas at the LBR in relation to macronutrients and other chemical parameters shows the same trends just described for heavy metals. Table 22 compares the mean concentrations of NO_3^- -N, P, TKN, conductivity, % organic matter, and pH of unsludged soils with the median and range for soils receiving sludge. As can be seen, all the parameters listed increased substantially after the sludge application except for pH which remained essentially unchanged. The principal reason that the pH did not decrease after sludge additions, as might be expected with the large additions of nitrogen contained in the sludge, is that these soils are strongly buffered with calcium carbonates.

Soils were sampled to a depth of 91 cm to investigate the rate or extent of leaching of pollutants such as nitrates, phosphorus, and heavy metals. Table 23 shows the median and range of nitrates and sodium bicarbonate extractable phosphorus to a depth of 91 cm.

Table 23 shows that phosphates are accumulating in the surface soil with no indication of leaching through the soil profile. Nitrate concentrations, while decreasing with depth, showed that substantial amounts had leached to a 91 cm depth. These results were expected since the amount of sludge applied annually (more than 67 tons/hectare) is far in excess of nitrogen requirements needed to grow winter wheat and other forages. The potential for groundwater pollution has been investigated by the U.S. Geological Survey (Robson, 1977) which concluded that nitrate concentrations in shallow wells downgradient from fields where sludge has been plowed into the soil were higher than background concentrations. No evidence of water quality degradation was found in deeper wells perforated into the bedrock formations.

Heavy metals (Zn, Cu, Ni, Cd, and Pb) were found to be concentrated in the surface soils with no indication of leaching below the depth of sludge incorporation. Tables 24 and 25 show the total metals concentrations of identical soils within close proximity of each other, except that the profile

TABLE 22. COMPOSITION OF LBR SURFACE SOILS FROM AREAS OF SLUDGE APPLICATION AND AREAS THAT HAVE NOT RECEIVED SLUDGE

Analysis	No Sludge Applications	Soils Receiving Sludge		
	Mean	Median	Range	
NO_3^- -N (ppm)*	2.4	232	4	- 860
TKN (ppm)*	1145.	1630	100	-5170
P (ppm)	8.	103	10	- 380
Organic Matter (%)	1.0	1.32	0.47-	3.7
Conductivity (mmhos/cm)	0.20	2.77	0.12-	5.61
pH	7.0	7.1	6.4 -	8.2

* NO_3^- and TKN expressed as ppm N.

TABLE 23. MEDIAN CONCENTRATION AND RANGE OF NITRATES AND PHOSPHORUS WITH SOIL DEPTH AT THE LBR

Element	$\mu\text{g/g}$ (Dry Weight)			
	Soils Receiving Sludge		Soils Receiving No Sludge	
	Depth (cm)	Median	Range	Mean
$\text{NO}_3^- - \text{N}^1$	0 - 30	232	<4 - 860	<2.4
$\text{NO}_3^- - \text{N}$	30 - 61	118	<4 - 350	<1
$\text{NO}_3^- - \text{N}$	61 - 91	61	<4 - 288	<1
p2	0 - 61	103	10 - 380	10
P	30 - 61	6	1 - 29	4
P	61 - 91	14	3 - 37	12

1. Nitrates expressed as ppm N.
2. NaHCO_3 extractable P.

shown in Table 25 had received a total application of 295 mT/ha of sludge since 1969. By comparing the two profiles it can be seen that apparently all of the metals that had been added with the sludge additions have remained within the depth of sludge incorporation.

According to sewage plant records the profile described in Table 25 had received 295 mT/ha (dry weight) of sludge. However, the fact that the surface horizon had only 91 ppm Zn (dry weight) would tend to indicate that less than this amount had actually been applied. This same trend (regardless

TABLE 24. CONCENTRATIONS OF TOTAL SOIL Zn, Cd, Cu, Ni, and Pb WITH SOIL DEPTH FROM AN UNSLUDGED AREA OF THE LBR¹

Depth (cm)	$\mu\text{g/g}$ (Dry Weight)				
	Zn	Cd	Cu	Ni	Pb
0 - 15	45.5	0.78	13.2	9.75	17.5
15 - 53	50.2	0.75	12.8	9.25	16.8
53 - 69	51.2	0.56	13.4	9.75	15.0
69 - 76	73.5	0.65	23.8	8.75	17.8

1. Taken from Profile VII described in Appendix B.

TABLE 25. CONCENTRATIONS OF TOTAL SOIL Zn, Cd, Cu, Ni AND Pb AFTER 295 mT/HA OF SLUDGE HAD BEEN APPLIED

Depth (cm)	$\mu\text{g/g}$ (Dry Weight)				
	Zn	Cd	Cu	Ni	Pb
0 - 23	90.9	1.35	27.1	15.0	31.8
23 - 46	53.8	0.22	14.5	7.50	11.5
46 - 69	61.0	0.28	13.2	8.50	13.0
69 - 91	70.5	0.72	11.2	5.75	19.5
91 - 114	74.0	0.42	11.8	5.75	15.5
114 - 147	56.0	0.18	17.0	4.25	12.0

1. Taken from Profile VIII described in Appendix B.

of metal) was noted in all the fields examined. The authors of this report can provide no explanation for this discrepancy, except that the amount of sludge actually applied has been grossly over estimated, or the bulk density of the soil is less than 1.

The effect of sewage sludge applications on the forages at the LBR are

TABLE 26. HEAVY METAL CONCENTRATIONS OF FORAGES FROM THE LBR

Element	$\mu\text{g/g}$ (Dry Weight)		
	No Sludge Applications		Sludge Applied
	Mean	Median	Range
Zn	11.7	75.8	37.2 - 153.
Cd	0.11	1.08	0.25 - 3.70
Cu	2.28	12.5	3.80 - 22.0
Ni	<0.57	2.80	0.25 - 15.5
Pb	2.3	0.75	<0.65 - 4.0
As	<0.25	<0.25	<0.25
Se	<0.35	<0.25	0.25
Hg	0.024	0.020	0.010 - 0.100

shown in Table 26. The mean heavy metal concentrations of forage tissues sampled in areas of the LBR which never received sludge applications are compared with the median and range of forage tissues taken from the 20 fields that had received varying applications of sewage sludge. The concentrations of Zn, Cd, Cu, and Ni increased significantly with the application of sludge. Concentrations of arsenic and selenium, which were generally below our detection limits of 0.25 ppm, did not increase to a measurable extent even with very high applications of sewage sludge. A much broader range of mercury concentrations were found in forages grown on the sludge amended soils; however, the median value (0.020 ppm) is equivalent to the mean of control forages. The concentration of lead in the soils receiving sludge increased significantly (Table 21), but the lead content of forages grown on these soils decreased significantly. The median value of forages grown on sludge amended soils was 0.75 ppm lead with a mean of 1.11 ppm, while forages from areas that never received sludge applications averaged 2.3 ppm (Table 26).

The decrease in lead tissue concentrations (significant at the 5% level) is most likely the result of a combination of factors. Ligands from the added organic matter may be chelating lead; thus, making it unavailable for plant uptake, or it may merely be a difference in plant species. Forages taken from areas of sludge incorporation were predominantly winter wheat that was green and actively growing, while forages taken from areas that never received sludge applications were pasture grasses (predominantly buffalo grass) which were growing poorly in a dry nutrient deficient soil. Because of the slow growth habits of the native pasture the decrease in lead may in fact be a dilution effect rather than an actual decrease in plant availability of soil lead. Another possibility which should not be overlooked is that the slower growing pasture grasses were exposed to air pollution for a much longer period of time. The grasses were washed in a dilute solution of lauryl-sulfate before analysis, but this may not have been sufficient to remove all the dirt adhering to and possibly enclosed in crevices and stomates of the dry pasture grass. Another possibility is that the large additions of phosphorus (Table 22) which accompany the sludge applications may be decreasing the ion activity of lead in the soils by precipitating the lead in a more insoluble form of lead phosphates. Abundant

TABLE 27. CORRELATION COEFFICIENTS OF DTPA SOIL EXTRACTABLES Zn, Cd, Cu, Ni, AND Pb COMPARED WITH THE METAL UPTAKE BY FORAGES

Element	Correlation Coefficient
Zinc	0.16
Cadmium	0.21
Copper	0.14
Nickel	0.17
Lead	0.04

TABLE 28. CORRELATION COEFFICIENTS OF TOTAL SOIL Zn, Cd, Cu, AND Pb COMPARED WITH THE METAL UPTAKE BY FORAGES

Element	Correlation Coefficient
Zinc	0.12
Cadmium	0.19
Copper	0.08
Nickel	0.06
Lead	0.07

phosphorus may also reduce lead transport from the roots of the grasses.

Each soil sample was extracted with DTPA (Lindsay and Norvell, 1978) in an attempt to correlate DTPA soil extractable Zn, Cu, Ni, Cd, and Pb with the plant uptake of these heavy metals. Correlation between DTPA extractable metals and the metal concentration of plant tissues was poor (Table 27). Correlations of total soil metals with forage metals concentrations were not any better (Table 28).

Soil pH also showed very poor correlation with plant uptake of metals, but this is probably a result of the narrow range of soil pH's encountered in these soils. All methods correlated very poorly with the actual uptake of metals into the plant tissues. When DTPA extractable metals were compared with the total metals analyses of the soils, the correlation was generally good (Table 29). This would indicate that the DTPA soil test could be used as a reliable index as to the total metal concentration of the soil.

TABLE 29. CORRELATION COEFFICIENTS OF DTPA SOIL EXTRACTABLES Zn, Cd, Cu, Ni, AND Pb COMPARED WITH THE TOTAL SOIL METALS

Element	Correlation Coefficient
Zinc	0.91
Cadmium	0.90
Copper	0.88
Nickel	0.46
Lead	0.61

Reasons for the lack of correlation of the DTPA extractable soil metals with plant uptake will not be dealt with in detail in this report; but, the reader is referred to articles by Viets and Lindsay (1973), Lindsay et al., (1966), Lindsay and Norvell (1969), and Norvell and Lindsay (1972). Possible explanations for the poor correlation between soil metal concentrations and plant uptake are that different grass species were examined which may display differences in uptake rates, and the tests were conducted over several soil types that varied in texture, pH, and possibly mineralogy. The DTPA soils test has demonstrated a remarkable ability to identify possible micronutrient deficiency problems and toxic levels of metals on uniform or acid soils; however, the lack of success seen here points to the need to develop a soil test which will give some reliable indication of metal availability over a broad range of calcareous soils when the metal levels are well above the deficiency level.

The problem of developing a soil test that will accurately reflect metal availability and plant uptake is further complicated by the fact that sewage sludge is not generally uniformly applied or mixed into the soil. The question as to how many soil samples from a particular site or field are needed to accurately reflect the nutrient or metal status of that field has been a question of debate for many years. With the need for environmental monitoring of sludge disposal sites and in other agricultural areas, this question is becoming of even greater import. The number of soil samples and the pattern of sampling has recently been examined by Ludwick et al. (1977) and Reuss et al. (1977) in relation to nitrates.

If the soils at the LBR are examined, using zinc as a specific example (other metals showed similar relationships), it can be shown that a fairly large number of samples need to be taken to accurately reflect the average heavy metal content. Table 30 shows the range of zinc concentrations that would be expected from the amount of sludge that has been applied to particular fields. The expected range has been calculated using the range of total soil zinc found in unsludged areas plus the normal range of zinc contained in the sludge (from Table 3). Also shown is the actual mean, the

TABLE 30. CONCENTRATIONS OF TOTAL SOIL ZINC COMPARED TO EXPECTED CONCENTRATIONS AFTER KNOWN SLUDGE ADDITIONS

Applied Sludge in mT/Hectare	No. of Samples	Mean µg/g	C.V. (%)	Expected Range µg/g
0	16	51	15	43 - 58
90 - 112	42	97	28	78 - 122
114 - 134	24	95	22	88 - 134
157	6	84	27	104 - 147
202	6	83	45	122 - 172

coefficient of variation, and the number of soil samples used to derive the mean and coefficient of variation. As can be seen by examining the coefficient of variation, the variability between samples becomes large with sludge additions, particularly at the highest rate of 202 mT/hectare. When 42 samples were averaged together the mean of the samples turned out to be in the middle of the expected range of zinc. When fewer samples were taken as in the 114-134 mT/hectare applications, the mean soil zinc fell into the expected range, but towards the lower end of the range. When only six samples were taken, as in the 157 and 202 mT/hectare fields, the mean underestimated the expected range considerably. These data tend to agree with the findings of Reuss et al. (1977) who recommended that the number of soil cores (for nitrate analysis) required for a 95% confidence interval of about $\pm 15\%$ of the geometric mean to be 82 samples per field; and 20 samples per field for a 90% confidence interval of about $\pm 26\%$ of the geometric mean. From these findings it could be concluded that a minimum of 20 composited soil cores from a particular field or site would be needed to accurately estimate the mean, median, or geometric mean of nitrates or metals in a field having received sludge applications.

Refractory Organics at the LBR and Control Ranch

The organic compounds examined in this study (Table 6) were chosen because these compounds represent a potential hazard to the human population. These pesticides are presently being examined by the U.S. Environmental Protection Agency in their National Human Monitoring Program for Pesticides. Of the 22 organic residues examined, only 9 (Table 31) were found in sludge or soil samples from the LBR. Plant tissues contained no detectable levels of the 22 residues examined.

Table 32 shows the concentration of organic residues that were detected in two types of composite sludge samples. The principal type of sludge that was disposed of at the LBR was raw primary plus waste activated sludge treated with ferric chloride and lime (F&L Cake). Small amounts of anaerobically digested primary sludge (DNS) were also disposed of at the LBR. The concentrations shown in Table 32 are on an as is basis and not expressed on a dry weight basis. The organic residues in DNS (1) and F&L cake are approximately five times greater if expressed on a dry weight basis. DNS (2)

TABLE 31. LIST OF ORGANIC RESIDUES IDENTIFIED IN SLUDGE OR SOIL SAMPLES

HCB	p,p'-DDT	PCB's (Archlor - 1254)
Chlordane	p,p'-DDD	
Oxchlordane	p,p'-DDE	
Dieldrin	alpha-BHC	

was air dried before analysis and represents the mean of 3 separate samples taken from a sludge that was used in the diet of beef cattle during feeding trials. By examining the concentration of residues determined in DNS (1) and DNS (2), it can be seen that there is considerable variation. Whether this variation is due to volatilization and degradation during the drying period of DNS (2) or if it represents a real variation is unknown. Residue levels of F&L cake are generally lower because the sludge is diluted 25 to 30% by ferric chloride and lime. By comparing the DNS sludges and the F&L cake sludge it can be seen that DDT is degraded to DDE during anaerobic digestion.

The concentrations of all residues detected in soil samples were at trace levels. Traces of p,p'-DDE, chlordane, dieldrin, and PCB's (AR-1254) were found in soil samples from the control ranch and the LBR, with no differences between areas receiving sludge and those that never received sludge. Thus, the application of sludge to the LBR did not increase the level of pesticides or PCB's over that normally found in these soils.

TABLE 32. CONCENTRATIONS OF REFRACTORY ORGANIC RESIDUES (PPB, WET WEIGHT) DETERMINED IN SLUDGE TYPES DISPOSED OF AT THE LBR

Compound	DNS(2) ¹	DNS(1) ²	F&L Cake ³
p,p'-DDT	<40.0	NF ⁴	202
p,p'-DDE	300	51	94
p,p'-DDD	497	11	65
alpha-BHC	16	NF	20
Dieldrin	86	101	35
HCB	NF ⁴	5	5
Oxychlordane	NF	94	NF
Chlordane	1535	1345	636
PCB's (Ar-1254)	4252	2570	751

1. DNS(2) - Air dried anaerobically digested primary (5% moisture content).
2. DNS(1) - Anaerobically digested primary sludge cake (80% moisture content).
3. F&L Cake - Raw primary plus waste activated secondary sludge treated with FeCl₃ and lime (80% moisture content).
4. NF - Not Found.

HEALTH AND TRACE ELEMENT LEVELS IN BEEF TISSUES FROM GRAZING SLUDGE AMENDED SOILS AT THE LOWRY BOMBING RANGE

ENCLOSED
 HEAD
 BEGIN
 SECTION
 HERE

Estimate of Soil Consumption by LBR Cattle

To make a rough estimate as to how much soil was being consumed by the LBR cattle, a number of soils and cattle feces were analyzed for Ti. Healy (1968, '70, '72, '74) had originally developed this concept from the fact that Ti is a relatively inert element that is not taken up into plant tissues; therefore, the concentration of Ti found in feces should reflect soil ingestion.

Sixteen surface soil samples from the LBR, half from areas receiving sludge applications and half from areas without sludge applications, were analyzed for Ti. The mean of all samples was found to be 0.66% TiO_2 (dry weight basis) with a standard deviation of 0.07%. The median and range were 0.65% and 0.52 to 0.74%, respectively.

Twenty-five cattle fecal chips were gathered from various areas of the LBR and analyzed for Ti. Only the inner portion of the collected fecal chips were analyzed to curtail contamination with dust or soil. The mean feces concentration was 0.10% TiO_2 with a standard deviation of 0.02%. The median concentration was 0.11% and the range was from 0.05 to 0.15%.

In calculating the average soil consumption by the LBR cattle we assumed a digestibility factor of 55% from the forages consumed by the cattle. From these admittedly rough estimates it can be calculated that the diet consumed by these cattle was approximately 8% soil; thus, these cattle have had ample opportunity to ingest significant quantities of sludge contaminants.

Health and Pathological Examination of Beef Cattle Grazing Sludge Amended Soils at the Lowry Bombing Range

During the fall of 1975 cattle from the LBR were compared with a control herd that grazed similar land located approximately 8 kilometers east of the LBR. Death losses from both herds were no greater than normal for the area, and neither herd appeared to have any greater incidence of disease than the other.

A group of 12 older cows from the LBR that had been exposed to sludge since the inception of sludge incorporation (1969) were slaughtered and compared with 6 control cows taken from the control ranch.

Both groups of animals were antemortem inspected by a veterinarian and all organs were examined postmortemly by a pathologist. Antemortem inspection revealed no overt symptoms of disease or pathology in either group. The cows from the LBR were assessed to be somewhat older than the cows from the control herd, but quantification of this age difference was

BOTTOM OF
 IMAGE AREA
 OUTSIDE
 DIMENSION
 FOR TABLES
 AND ILLU-
 STRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

Typing Guide Sheet

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTION
HERE

impossible because there were no records of birth dates of the cattle from the LBR. All animals were judged to be over 4 years of age. Postmortem inspection of internal organs showed no significant differences between the two groups. Observed lesions and abscesses were typical of older range cattle and were not overt symptoms caused by sludge components in the diet.

Concentration of Trace Metals in Tissues of Range Cattle Grazing Sludge Amended Soils at the Lowry Bombing Range

The mean trace element concentrations of kidney, liver, bone, and muscle tissues for the two groups of animals is shown in Table 33. Arsenic (As) ranged from less than 0.02 ppm to 0.03 ppm (dry weight basis) with no significant differences between the tissues examined. The As levels found were neither elevated nor depressed from what would be expected in normal animals (Underwood, 1977).

Mercury (Hg) concentrations were higher in kidney tissues than other tissues examined. The control group of cattle showed significantly higher renal Hg concentrations with a mean of 0.12 ppm compared to 0.06 ppm in the sludge exposed cattle. However, the Hg levels determined in both groups of animals were not out of the range of concentrations that would be expected in normal animals (Underwood, 1977).

Molybdenum (Mo) concentrations were significantly different between the two groups of animals, but as expected, were significantly higher in liver tissues. The mean Mo concentrations of 2.7 and 3.2 ppm (dry weight basis) are within the normal range of 2 to 4 ppm normally found in cattle.

REMAIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA:
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

TABLE 33. MEAN CONCENTRATION OF TRACE ELEMENTS IN TISSUES OF SLUDGE EXPOSED LBR RANGE CATTLE (E) AND CONTROL RANCH CATTLE (C)

	$\mu\text{g/g}$ (Dry Weight)									
	As	Hg	Mo	Ni	Pb	Se	F	Cd	Cu	Zn
Kidney-C	0.03	0.12	1.7	<0.5	0.9	5.2	ND ²	5.9	16.3	76.
Kidney-E	0.02	0.06	1.6	<0.6	0.8	5.1	ND	16.*	16.1	93.*
Liver-C	0.02	0.02	3.2	<0.8	0.25	1.7	ND	0.8	19.	113.
Liver-E	0.02	0.02	2.7	<0.3	0.26	1.0*	ND	1.4	4.6*	129.
Bone-C	<0.03	<0.02	0.15	<0.7	1.5	0.06	550	0.016	0.5	58.
Bone-E	<0.02	<0.02	0.35	<0.5	3.1	0.1	650	0.011	1.3	68.*
Muscle-C	<0.02	<0.004	<0.03	<0.2	<0.2	0.67	ND	<0.01	2.9	262.
Muscle-E	<0.02	<0.004	<0.05	<0.4	<0.2	0.81	ND	<0.039	2.5	247

1. Values followed by * are significantly different at the 5% level (Student's t-test).
2. ND - Not Determined.

BEST
FIRST
LINE OF
TEXT
HERE

Tissue concentrations of Ni were generally below detection limits with little difference between tissues of the two groups of animals.

DROPPED
HEAD
BEGIN
SECTION
HERE

Lead concentrations were highest in bone tissues of both groups of animals with the sludge exposed group showing higher, but not significantly higher, concentrations than the control group. This would be expected if the cattle from the LBR were older than the control cattle since lead accumulates in bones with age (Schroeder and Tipton, 1968). The Pb concentrations in liver, kidney, muscle, and bone tissues of both groups of animals were well within the concentration range considered normal for cattle (Allcroft, 1951; Doyle, 1979).

A significant difference was found between the Se concentrations of liver tissues from the two groups of cattle. The sludge exposed LBR cattle had significantly less Se with a mean of 1.0 ppm (dry weight basis) as compared to 1.7 ppm in the control ranch cattle. Selenium levels in other tissues were not significantly different between the two groups. Kidney tissues displayed the highest concentration of Se relative to other tissues with a mean of just over 5 ppm (dry weight basis). The Se levels determined in the tissues of both groups of animals are within the range of expected values for cattle (Underwood, 1977).

Levels of F in bone tissues increase in proportion to the amount of F in the diet, the form of F, and the age of the animal (Underwood, 1977). The F concentrations of bone tissues between the two groups of cattle were not significantly different, although the mean level of F in bone of the sludge exposed cattle was higher at 650 ppm (dry weight basis) than the control herd at 550 ppm. These values are within the range that is commonly found in farm animals (Lloyd, 1970; Underwood, 1977).

The Cd concentration of kidney tissues was significantly higher in the sludge exposed cattle than the control cattle. Liver concentrations of Cd were also higher in the sludge exposed animals; however, this was not statistically significant at the 5% level. The Cd concentration of kidneys has been shown to increase with age (Piscator, 1974, 1972). The differences in Cd concentrations between the two groups of animals is therefore most likely a result of age differences between the two groups rather than an effect of sludge exposure. Evidence to support this concept is that one of the control animals contained a renal Cd concentration of 16 ppm Cd (dry weight basis), which was the mean of the sludge exposed group. The examination of cattle of known age and no sludge exposure (to be discussed in the next section) tends to verify the finding that increased renal Cd was an age effect rather than a sludge effect.

Possible interactions with Cd and other heavy metals in these animals involves Cu and Zn. A number of investigators have shown that with increasing tissue Cd there is an increase in tissue Zn (Powell et al., 1964; Piscator, 1972, 1974; Elinder et al., 1977), while at high levels of Cd intake there is generally a decrease in Zn concentration of bone and muscle tissue (Mills and Dalgarno, 1972; Anke et al., 1970; Jacobs et al., 1973). Concurrent with increasing concentrations of Cd in the kidney and liver, there is usually a decrease in Cu concentrations of liver tissues (Mills and

BELOW
LAST LINE
OF TEXT

BOTTOM OF
MARGIN AREA
OUTSIDE
TEXT AREA
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

Dalgarno, 1972; Anke et al., 1970; Doyle and Pfander, 1975).

DROPPED
HEAD
BEGIN
SECTION
HERE

The sludge exposed animals showed a significant increase in Zn concentrations of kidney and bone, while other tissues were not significantly different between the two groups. This would be expected if the LBR cattle were significantly older than the control ranch animals (Elinder et al., 1977, 1978). It is important to note that there was not a decrease in the Zn content of bone or muscle tissues which might indicate excessive levels of Cd ingestion.

Underwood (1977) states that the normal range of hepatic Cu is 100 to 400 ppm (dry weight basis). In most animal species the Cu concentrations in liver tissues decrease with age, however, sheep and cattle are supposed to be the exceptions, with increasing Cu concentrations with age in sheep and little or no change throughout the life of cattle (Underwood, 1977). Cunningham (1946) examined the Cu concentrations of liver tissues taken from 41 copper deficient mature cattle and found a mean concentration of 11.5 ppm (dry weight) with a range of 3 to 32 ppm. On this basis, both groups of cattle in this study displayed lower than normal liver Cu levels, with a mean of 18.6 ppm (dry weight basis) for the controls and 4.6 ppm for the sludge exposed animals. These low Cu levels would tend to indicate that these animals had been on a Cu deficient diet, however, there has been no indication of Cu deficiency in the area and these cattle did not appear to be suffering from Cu deficiency. Information regarding normal Cu liver concentrations is limited; thus, the levels reported here may not be as low as thought at first glance. The livers of normal adults of many other species contain 10 to 50 ppm Cu (Underwood, 1977).

The sludge exposed animals contained significantly less liver Cu at 4.6 ppm (dry weight basis) than the control animals at 18.6 ppm. An obvious conclusion to draw from the work of Mills et al. (1972), Anke (1970), Doyle et al. (1975), and others is that the increased Cd in the kidney is reducing hepatic Cu concentrations. This indeed may be the case; however, if hepatic Cu were being reduced because of excessive Cd, a reduction in bone Cu might also be expected, which was not seen here. The bone Cu level of the sludge exposed group is significantly higher at the 10% level of probability, indicating that there was no Cu depletion of bone tissue taking place.

The increases in Zn and Cd in kidney tissues, and decrease in liver Cu may indicate interactions due to sludge exposure, or may be the result of increases and decreases in trace elements as a result of normal aging processes and environmental exposure to these elements. A study to be discussed later, involving range cattle of known age and no exposure to sewage sludge will indicate that the LBR cattle did not have elevated levels of renal Cd nor unusually low hepatic Cu concentrations.

BEGIN
LAST LINE
OF TEXT

There were no significant differences in trace metal concentrations of muscle tissue between the two groups of cattle. Cadmium and lead concentrations were generally below detection limits indicating that muscle tissues were well protected from accumulations of cadmium, lead, and all other elements examined.

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

3 1/2"

60

PAGE NUMBER

Refractory Organic Residues in Tissues of Cattle Grazing the LBR and Control Ranch

Of the tissues examined (fat, liver, kidney, muscle, bone, brain, lung, spleen, and blood) only fat, showed any detectable levels of the refractory organic residues examined. Measurable levels of alpha-BHC and traces of HCB, p,p'-DDE, dieldrin, and AR-1254 were found in fat tissues of range cattle grazing both the LBR and the control ranch (Table 34). Fat tissues from the control group of cows contained significantly higher concentrations of alpha-BHC than the sludge exposed cows.

Investigation of Heavy Metals Content in Kidney and Liver Tissues From Cattle of Known Age and No Previous Exposure to Elevated Levels of Heavy Metals

The LBR study of heavy metals in cattle tissues revealed that the LBR cattle had significantly higher renal Cd levels than the control group of animals, but the unanticipated differences in age between the sludge exposed animals and the control group made it impossible to separate these differences on the basis of sludge exposure or an age effect. Therefore, another study was undertaken to examine the effects of age on metal concentrations in animals that had never been exposed to sewage sludge or other known sources of elevated levels of heavy metals.

This new control study consisted of 25 range cattle from eastern Colorado, 4 range cows from eastern Wyoming, 15 dairy cows from Colorado State University, and 12 steers used as controls in sludge feeding trials. The 29 range cows varied in age from 5 to 15 years, the steers were approximately 18 months old at the time of slaughter, and the dairy cows ranged in age from 2 to 8 years (table 35).

Table 36 shows the mean concentrations of Zn, Cu, Cd, Pb, and Fe in kidney and liver tissues from the known age dairy cattle and range cattle. These two groups of animals showed significant differences in Cu and Cd concentrations in kidney tissues and significant differences in Cu

TABLE 34. CONCENTRATION OF REFRACTORY ORGANIC RESIDUES DETERMINED IN FAT TISSUES OF RANGE CATTLE FROM THE LBR AND THE CONTROL RANCH

Source	Animals Sampled	ppb (Wet Weight) ¹				
		HCB	Alpha-BHC	p,p'-DDE	Dieldrin	AR-1254
LBR	12	<10	10a ²	10	10	500
Control	6	<10	30 b	10	10	500

1. Mean % moisture of fat tissue = 16%

2. Significantly different at the 1% level (Student's t-test).

TABLE 35. AGE DATA OF DAIRY AND RANGE CATTLE HAVING NEVER BEEN EXPOSED TO SEWAGE SLUDGE WHICH WERE USED IN THE "ANIMAL AGE" STUDY

	<u>Number</u>	<u>Mean Age</u>	<u>Median Age</u>	<u>Age Range</u>
Range Cattle of Known Age	29	10	10	5 - 15
Dairy Cattle of Known Age	15	4	4	2 - 8

concentrations in liver tissues. The Cu concentrations of liver tissues from the dairy cattle were for the most part an order of magnitude greater than

TABLE 36. MEAN CONCENTRATION OF TRACE METALS DETERMINED IN KIDNEY AND LIVER TISSUES FROM CONTROL CATTLE OF KNOWN AGE

<u>Tissue and Element</u>	<u>µg/g (Dry Weight)</u>	
	<u>Range Cattle of Known Age</u>	<u>Dairy Cattle of Known Age</u>
Kidney:		
Zn	88.4 ± 17.6 ¹	96 ± 46
Cu	15.8 ± 3.2a ²	21.7 ± 2.6b
Cd	13.4 ± 8.0a	2.8 ± 1.7b
Pb	3.6 ± 1.7	1.9 ± 1.3
Fe	225 ± 73	293 ± 64
Liver:		
Zn	132 ± 25	118 ± 26
Cu	21.3 ± 32.9a ³	216 ± 96b
Cd	1.06 ± 0.6	0.74 ± 0.9
Pb	1.8 ± 1.1	1.0 ± 1.0
Fe	256 ± 99	250 ± 72

1. Mean ± standard deviation.

2. Values followed by different letters are significantly different at the 1% level using Student's t-test.

3. If the 4 range cows from Wyoming are excluded from this analysis the mean of the 25 Colorado range cows becomes 9.5 ± 3.18 µg/g Cu. The difference between range and dairy cattle is still significant.

liver tissues from the range cattle. While there is a large age difference between these two groups of cattle, the differences could not be accounted for by decreasing Cu concentrations with age; thus, the different Cu status of these two groups is probably diet related. The dairy cattle may have received dietary trace element salt supplements (although this could not be confirmed) which would have included Cu, and the range cattle would most likely not have received such dietary supplements. Renal Cd concentrations between the dairy cattle and the range cattle were significantly different. However, when animals of similar age (4 animals from the dairy group and 13 animals from the range group) were compared, there were no significant differences; thus, the Cd differences between the two groups may have been an age effect.

Comparisons shown in Table 37 are between the 29 range cows in the "animal age" study and the sludge exposed LBR range cows discussed previously. The data show that there were no significant differences in renal Cd concentration between the sludge exposed cattle and the control group of animals. Examination of Zn and Pb also showed no significant differences. However, there was a significant difference between hepatic Cu concentrations. The LBR cattle had significantly less hepatic Cu. Reasons as to why the sludge exposed LBR cattle had lower hepatic Cu concentrations are unclear. This may be a sludge effect; however, this seems unlikely. If the LBR cattle were consuming significant quantities of sludge, hepatic Cu concentrations would most likely rise from the increased dietary Cu. Since renal and hepatic Zn and Cd concentrations are similar, it is unlikely that

TABLE 37. MEAN TISSUE CONCENTRATION OF CONTROL RANGE CATTLE (KNOWN AGE) AND SLUDGE EXPOSED LBR CATTLE

Tissue	Animals Sampled	µg/g (Dry Weight)				
		Cd	Cu	Zn	Pb	Fe
Kidney:						
Control (Range)	29	13.4a ¹	15.8a	88.4a	3.6a	225
LBR	12	16 a	16.1a	93 a	0.8a	ND ²
Liver:						
Control (Range)	29	1.1a	9.5a ³	132a	1.8a	256
LBR	12	0.8a	4.6 b	129a	0.3a	ND

1. Values followed by different letters within a column are significantly different at the 1% level (Student's t-test).
2. ND - Not Determined.
3. This Cu value excluded the 4 range cows from Wyoming (see table 35).

interactions between these metals would be affecting hepatic Cu. The most probable explanation is that the low hepatic Cu concentrations are a reflection of lower dietary intake of Cu.

The effect of age on renal concentrations is shown in Figure 2. Data shown in Figure 2 represents all animals of known age used in the "animal age" control study. As can be seen, the Cd concentrations significantly increased with age. The range of renal Cd concentrations was from 0.83 ppm in a 2 year old dairy cow to 31.2 ppm in a 13 year old range cow.

It was originally hypothesized that hepatic Cu concentrations might decrease as cattle age (a dilution effect of the growing liver). This would help explain much of the data showing the LBR and control cattle with extremely low hepatic Cu concentrations. Figure 3 shows mean hepatic Cu concentrations of the control animals in relation to age. It appears that hepatic Cu concentrations at first increase and then drop off rather dramatically when the cattle reach age 6. Most likely the increases and decreases seen in Figure 3 were the results of diet rather than age. The first group of 12 animals represent young steers that were on a feed lot diet with mineral supplements. The second and third group of animals up to age 5.5 were for the most part dairy cows, which may have received trace mineral supplements with their feed. The last five groups, all showing low hepatic Cu concentrations were range cows that grazed arid pastures which may have contained less Cu. There appears to be too great a decrease in hepatic Cu concentrations between 4 and 6 year old animals to be explained

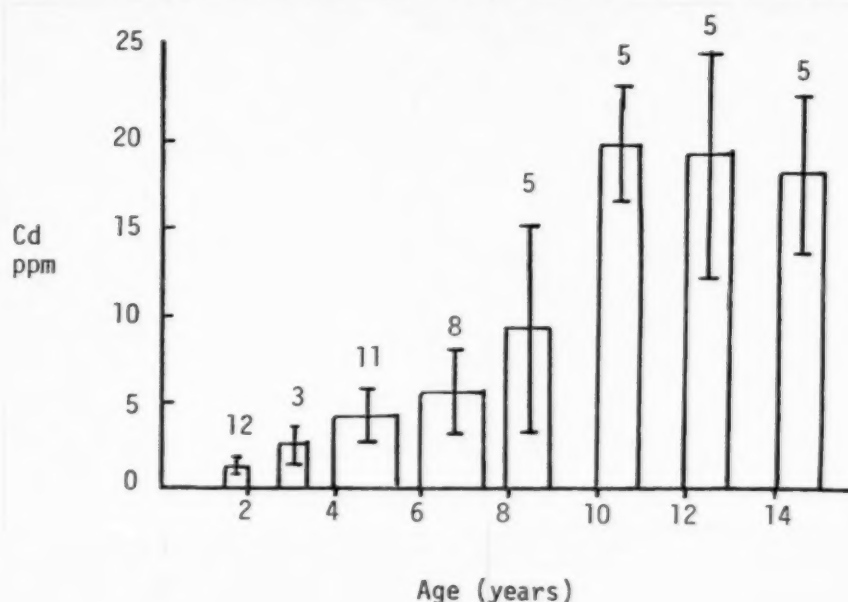


Figure 2. Mean concentration of Cd in kidney tissues (dry weight) as a function of cattle age. Numbers above bars represent number of animals for each age group. Tick marks represent standard deviation for each group.

on the basis of age. Therefore, the most likely explanation rests with diet.

A number of investigators (Schroeder et al., 1967; Anke et al., 1970; Piscator, 1974) have shown that increases in kidney cortex Cd have been accompanied by increases in cortex Zn concentrations. Elinder and Piscator (1977, 1978) found that Zn increased in kidney cortex of horses equimolarly with increasing Cd. It was postulated that the increased Zn was due to renal metallothionein, which contains equimolar amounts of Cd and Zn. However, the cattle examined in this study did not show this relationship. The older range cows which generally had higher renal Cd levels did show slightly higher average renal Zn levels, but the differences between the groups was far from significant.

EFFECTS OF DIRECT INGESTION OF METRO DENVER SLUDGE BY BEEF CATTLE

Performance of Cattle Fed Metro Denver Sludge

As described previously, Metro Denver anaerobically digested primary sludge was added to a feed lot diet of beef cattle to produce 3 rations consisting of approximately 0, 4, and 12% sludge (dry weight basis). The cattle (6 per group) were maintained on this diet for 94 days.

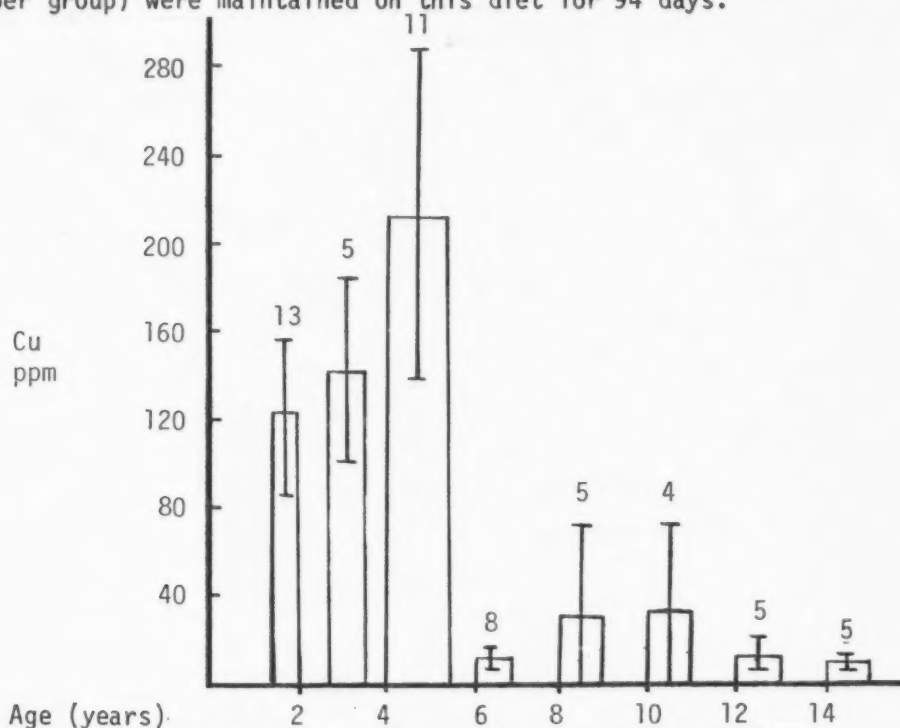


Figure 3. Mean concentration of Cu in liver tissues (dry weight) as a function of cattle age. Numbers above bars represent number of animals for each group. Tick marks represent standard deviation for each group.

TABLE 38. AMOUNT OF FEED AND SLUDGE CONSUMED (6 STEERS/TREATMENT) AND THE OVERALL % FEED EFFICIENCY FROM THE SLUDGE TREATMENTS (METRO DENVER SLUDGE)

Diet	Kg Total Wt. Gain	Kg (Dry Wt.) Dry Matter Consumed	Kg (Dry Wt.) Sludge Consumed	Feed ¹ Efficiency
0%	133	5,197	0	2.6%
4%	119	5,404	199	2.2%
12%	106	5,217	587	2.0%

1. Feed Efficiency (gain:feed ratio).

Table 38 shows the percent of sludge consumed by the cattle, the amount of sludge and feed consumed over the 94 day feeding period, and the feed efficiency from the total diet consumed. The effect of sludge additions on weight gain is shown in Table 39. The sludge fed animals gained significantly less weight than the control animals. The feed efficiency of the sludge fed steers averaged 2.2 and 2.0% for the 4 and 12% sludge diets which was lower than the 2.6% observed with the controls. The gain to feed ratio calculated from non-sludge components, however, was essentially equal for the three groups. The expected gains calculated from feed consumption agreed well with observed gains when the sludge component was assumed to have no energy value (Table 39). Results from this study would indicate that anaerobically digested primary sludge had no positive or negative effects on general performance of beef cattle other than to act as a diet diluent. All animals remained healthy throughout the experiment and no signs of pathology related to the sludge component of the diet was evident.

TABLE 39. THE EFFECTS OF SLUDGE (METRO DENVER) IN DIET UPON FEED LOT CATTLE PERFORMANCE

Diet	Mean Body Wt. (Kg)				Mean Total Gain (Kg)	Mean Daily Gain		
	After Days on Test					Observed	Expected ¹	Observed/ Expected
	0	35	81	94				
0% Sludge	344	386	462	477a ²	133a	1.42	1.37	1.03
4% Sludge	344	386	446	463ab	119ab	1.27	1.39	.91
12% Sludge	344	379	454	450 b	106 b	1.12	1.18	.95

1. Expected gain calculated according to NRC (1970) from feed consumption assuming no energy contribution from sludge.
2. Values followed by different letters are significantly different at the 5% level (Student's t-test).

Trace Element Concentration of Beef Cattle Tissue Fed Metro Denver Sludge

As expected, the tissues that retained the highest concentrations of all metals except Zn were kidney and liver. Table 40 shows the mean concentrations of Zn, Cd, Cu, Pb, and Hg in kidney and liver tissues.

Zinc concentrations of kidney tissues were unaffected by increasing the Zn concentrations of the diet through sludge additions. Renal Cd concentrations increased significantly when the diet contained 4% sludge. Renal Cd did not increase when diets containing 4 and 12% sludge were compared. This fact is significant in that it indicates that the accumulations of renal Cd was not entirely dose related. A number of investigators (Piscator, 1972, 1974; Elinder et al., 1976; Cousins et al., 1973) have shown that Cd accumulates in a linear fashion until a threshold level in the kidney is reached, at which point Cd concentrations tend to level off, but this has not been shown to occur at the low levels of renal Cd displayed in this study.

Hepatic Zn concentrations increased significantly when the 12% sludge diet was consumed. However, the range of liver Zn concentrations observed were not out of the ordinary, and this effect was not seen in two additional sludge feeding trials to be discussed later; thus, it is doubtful that this increase has any biological significance. Like renal Cd concentrations, hepatic Cd significantly increased in response to the 4% sludge diet, but no further increase was evident from ingestion of the 12% sludge diet.

TABLE 40. MEAN CONCENTRATION OF Zn, Cd, Cu, Pb, and Hg IN KIDNEY AND LIVER TISSUES OF BEEF CATTLE FED A DIET CONTAINING 0, 4, and 12% METRO DENVER SLUDGE FOR 3 MONTHS

Tissue Type & % Sludge in Diet	Animals Sampled	$\mu\text{g/g}$ (Dry Weight)				
		Zn	Cd	Cu	Pb	Hg
Kidney - 0	5	84a ¹	1.1a	17a	0.9a	0.1 a
Kidney - 4	5	ND ²	2.5 b	18a	12.2 b	0.45 b
Kidney - 12	5	82a	2.4 b	15 b	15.8 c	0.9 c
Liver - 0	5	87a	0.2a	124a	0.2a	0.01a
Liver - 4	5	99ab	0.5 b	260 b	3.3 b	0.06 b
Liver - 12	5	101 b	0.4 b	240 b	4.6 c	0.14 c

1. Values followed by different letters are significantly different at the 5% level (Student's t-test).
2. ND - Not Determined.

A small, but statistically significant, decrease in renal Cu concentrations occurred when the 12% sludge diet containing the highest concentration of Cu was fed, but not when the 4% sludge diet was fed. The 2 ppm decrease of Cu in kidney was statistically significant, but it is doubtful that this has biological significance. Hepatic Cu, on the other hand, increased significantly when cattle consumed the 4% sludge diet, but no further increase was seen when the 12% sludge diet was fed. In fact, a slight decrease (not statistically significant) in hepatic Cu was evident when the 12% sludge diet was fed.

Concentrations of renal and hepatic Pb and Hg appear to be dose related with increasing concentrations retained in these tissues as dietary Pb and Hg were increased.

Concentrations of As, Mo, Ni, and Se determined in kidney and liver tissues are shown in Table 41. Arsenic concentrations increased in both the liver and kidney due to sludge additions. Molybdenum concentrations in both the kidney and liver decreased significantly when the high sludge diet was fed. The interrelationships between Cu, Mo, and SO₄ have been known for some years (Dick, 1952; Goodrich and Tillman, 1966). The reductions in hepatic and renal Mo seen here may have been the result of increased dietary sulfate that most likely occurred when increasing amounts of sludge were added to the diet. Concentrations of Ni and Se in liver and kidney tissues were not affected by the sludge additions to the diet.

Table 42 shows the concentrations of Zn, Cd, Cu, Pb, Hg, and F found in bone, muscle, and blood samples. Zinc concentrations in bone, muscle, and

TABLE 41. MEAN CONCENTRATIONS OF As, Mo, Ni, AND Se IN KIDNEY AND LIVER TISSUES OF BEEF CATTLE FED A DIET CONTAINING 4 AND 12% METRO DENVER SLUDGE FOR 3 MONTHS

Tissue Type and % Sludge in Diet	Animals Sampled	µg/g (Dry Weight)			
		As	Mo	Ni	Se
Kidney - 0	5	0.05a ¹	1.4a	<0.2a	5.4a
Kidney - 4	5	0.11 b	ND ²	ND	ND
Kidney - 12	5	0.17 b	0.94 b	<0.5a	5.4a
Liver - 0	5	0.02a	2.6a	<1 a	1.3a
Liver - 4	5	0.06 b	ND	ND	1.7a
Liver - 12	5	0.06 b	2.0 b	<0.2a	1.7a

1. Values followed by different letters are significantly different at the 5% level (Student's t-test).
2. ND - Not Determined.

blood samples were unaffected by sludge additions. Cadmium concentrations were generally below detection limits in all 3 tissues, indicating a remarkable ability of the kidney and liver to sequester Cd and prevent it from entering muscle tissues; thus, muscle tissues seem to be well protected from accumulations of Cd.

Copper concentrations in blood, bone, and muscle tissue were not altered by the sludge additions to the diet. Lead concentrations in bone and blood samples increased significantly with sludge additions to the diet, while no increase in Pb concentration was evident in muscle tissues. Mercury concentrations of bone and blood samples were not affected by the sludge treatments; however, muscle tissues showed a statistically significant rise in Hg concentrations when the 12% sludge diet was consumed. The Hg increase in muscle tissue was small; and while this was statistically significant, it is doubtful that this apparent increase was biologically significant.

The only tissue that F was determined in was bone tissue, which showed a significant increase due to sludge ingestion (Table 42).

Concentrations of As, Mo, Ni, and Se in bone, muscle, and blood tissues are shown in Table 43. Bone tissues showed an apparent increase in Mo concentrations when the 12% sludge diet was consumed by the cattle while As,

TABLE 42. MEAN CONCENTRATIONS OF Zn, Cd, Cu, Pb, Hg, AND F IN BONE, BLOOD AND MUSCLE TISSUES OF BEEF CATTLE FED 0, 4 and 12% METRO DENVER SLUDGE FOR 3 MONTHS

Tissue Type and % Sludge in Diet	Animals Sampled	μ g/g (Dry Weight)					
		Zn	Cd	Cu	Pb	Hg	F
Bone - 0	4	64a ¹	<0.004a	1.8a	0.75a	<0.01a	200a
Bone - 4	5	ND ²	ND	ND	3.7 b	ND	ND
Bone - 12	4	58a	<0.005a	1.4a	11. c	<0.01a	400 b
Muscle - 0	5	253a	<0.006a	2.4a	<0.2a	<0.004a	ND
Muscle - 4	5	ND	ND	ND	ND	<0.004a	ND
Muscle - 12	6	273a	<0.01a	2.4a	<0.2a	<0.012 b	ND
Blood - 0	4	15a	<0.006a	6.3a	0.12a	<0.002a	ND
Blood - 12	4	19a	<0.009a	6.7a	0.82 b	<0.006a	ND

1. Values followed by different letters are significantly different at the 5% level (Student's t-test).
2. ND - Not Determined.

TABLE 43. MEAN CONCENTRATIONS OF As, Mo, Ni, and Se IN BONE, BLOOD, AND MUSCLE TISSUES OF BEEF CATTLE FED 0, 4, and 12% METRO DENVER SLUDGE FOR 3 MONTHS

Tissue Type and % Sludge in Diet	Animals Sampled	$\mu\text{g/g}$ (Dry Weight)			
		As	Mo	Ni	Se
Bone - 0	4	<0.02a	0.07a	<0.02a	0.09a
Bone - 4	5	ND ²	ND	ND	ND
Bone - 12	4	<0.02a	0.17 b	<0.2 a	0.09a
Muscle - 0	5	0.02a	<0.03a	0.2 a	0.81a
Muscle - 4	5	0.02a	ND	ND	ND
Muscle - 12	6	0.05 b	<0.04a	<1. a	0.85a
Blood - 0	5	0.02a	<0.08a	<0.4 a	1.3a
Blood - 12	4	<0.02a	0.27 b	0.6 a	1.7a

1. Values followed by different letters are significantly different at the 5% level (Student's t-test).
2. ND - Not Determined.

Se, and Ni remained unchanged. Arsenic increased in muscle tissues at the high rate of sludge ingestion, while Mo, Ni, and Se remained unchanged. Blood samples showed an increase in Mo, while levels of As, Ni and Se were not significantly changed.

Mean concentrations of trace elements found in spleen, lung, brain, and fat tissues are shown in Table 44. Of the trace elements examined in these tissues, the only metal that showed an apparent change due to sludge ingestion was Pb concentration in lung tissue, which appeared to increase when the 12% sludge diet was consumed.

While many of the trace element concentrations increased in a number of tissues examined, none of the tissues displayed levels that would be considered abnormally high or low for normal healthy animals. None of the heavy metals were transferred to any significant extent to muscle or fat tissues, demonstrating that the major edible portions of cattle were protected from heavy metal accumulations.

TABLE 44. MEAN CONCENTRATION OF TRACE ELEMENTS IN SPLEEN, LUNG, BRAIN, AND FAT TISSUES OF BEEF CATTLE FED 0 AND 12% METRO DENVER SLUDGE FOR 3 MONTHS

Tissue Type & % Sludge in Diet	Animals Sampled	g/g (Dry Weight)								
		As	Cd	Cu	Hg	Mo	Ni	Pb	Se	Zn
Spleen - 0	3	0.02	0.024	3.1	0.01	1.2	0.4	0.10	1.35	83
Spleen - 12	3	0.03	0.014	3.1	0.03	3.4	0.3	1.5	1.27	88
Lung - 0	3	0.02	0.014	5.7	0.01	0.7	0.9	0.14	1.23	73
Lung - 12	3	0.03	0.04	5.8	0.03	0.9	0.4	0.38*	1.23	77
Brain - 0	4	<0.02	<0.005	9.6	<0.02	0.10	<0.6	0.2	0.96	41
Brain - 12	5	<0.02	<0.006	8.6	<0.007	0.09	<1.	0.04	0.87	41
Fat - 0	1	<0.02	<0.005	<0.5	<0.02	0.04	<0.5	0.04	0.10	1.9
Fat - 12	1	<0.02	<0.004	<0.5	<0.02	0.02	<0.5	0.16	0.08	0.1

*Significantly different at the 5% level (Student's t-test)

Refractory Organic Residues in Tissues of Cattle Fed Metro Denver Sludge as a Portion of Their Diet

PROOF
HEAD
BEGIN
SECTION
HERE

Pesticides and persistent organics can enter the body by a number of pathways, but the most prevalent is through direct ingestion, generally from contaminated food. When sewage sludge is used as an agricultural fertilizer there is a potential hazard that pesticides which might be found in sewage sludge, could enter the food chain.

Tissues from the cattle fed 0, 4, and 12% anaerobically digested Metro Denver sewage sludge were examined for refractory organics. Samples of fat, kidney, brain, blood, liver, and muscle tissues were analyzed for the 22 refractory organics listed in Table 6. Sewage sludge and feed components were also analyzed for the same organics. Concentrations of the various refractory organics determined in the sludge, silage, feed and water is shown in Table 45.

Traces of p,p'-DDE, dieldrin, and AR-1254 were found in livers of sludge treated animals while no residues were detected in livers from control animals. Traces of alpha-BHC, p,p'-DDE, and dieldrin were found in muscle tissues of both control and sludge treated animals. Kidney, brain, and blood samples contained no detectable residues in any of the treatments.

TABLE 45. RANGE OF ORGANIC RESIDUES FOUND IN (ppb WET WEIGHT) SLUDGE, SILAGE, FEED, AND WATER OF METRO DENVER SLUDGE FEEDING TRIAL

Compound	Sludge ¹	Feed ²		Silage ³	Water
		0% Sludge	12% Sludge		
HCB	ND ⁴ - <4.0	ND	<10	ND	ND
p,p'-DDE	30 - 70	ND	<20	ND	<0.24
p,p'-DDD	ND - 30	ND	ND	ND	ND
Dieldrin	80 - 140	ND	ND	ND	ND
Chlordane	1160 - 1600	ND	160 - 170	ND	ND
Oxychlordane	80 - 116	ND	<10	ND	ND
AR-1254	2150 - 2850	ND	<500	<40	ND

1. Percent moisture of sludge ~30%.
2. Percent moisture of feed ~10%.
3. Percent moisture of silage ~67%.
4. ND - Not Detected

BOTTOM OF
PAGE AREA
OUTSIDE
DIMENSIONAL
FOR TABLES
AND ILLUSTRATIONS

100%
 100%
 100%
 100%
 100%

TABLE 46. MEAN CONCENTRATION OF p,p'-DDE, DIELDRIN, AND AR-1254 IN FAT TISSUES OF BEEF CATTLE FED METRO DENVER SEWAGE SLUDGE¹

% Sewage Sludge In Diet	µg/kg (Dry Weight)		
	p,p'-DDE	Dieldrin	AR-1254
0	<20	<10	<500
12	56	40	1000

1. Mean % moisture of fat tissue was 11%. Mean % lipid of all fat tissues was 70%.

Fat tissues were the only tissues that showed a significant increase in any of the persistent chlorinated hydrocarbons due to sludge additions to the diet. Trace levels of HCB, alpha-BHC, beta-BHC, lindane, oxychlordane, and TNC were found in fat tissues of both control and animals receiving sludge amended diets; however, significant increases of p,p'-DDE, dieldrin, and AR-1254 resulted from sludge ingestion (Table 46).

All of these organics are below tolerance levels that have been established by FDA. At the present time, tolerance levels for TNC and HCB in red meat have not been set.

EFFECTS OF DIRECT INGESTION OF FT. COLLINS SLUDGE BY BEEF CATTLE

Three-Month Ingestion Period

Performance of Cattle Fed Ft. Collins Sludge for 3 Months--

As described previously, Ft. Collins anaerobically digested sewage sludge was added to a feed lot diet of steers to produce 2 rations consisting of approximately 0 and 12% sludge (dry weight basis). The cattle (6 per group) were maintained on this diet for 106 days.

The principal differences between the Metro Denver sludge and the Ft. Collins sludge were that the Ft. Collins sludge contained 98 ppm Cd and 1700 ppm Cu (dry weight basis), whereas, the Metro Denver sludge contained 21 ppm Cd and 710 ppm Cu (dry weight basis) (Table 47).

The feeding trials began on November 12, 1976, and were terminated 106 days later on February 23, 1977. Table 48 shows the amount of sludge and feed consumed over the 106 day feeding period and the feed efficiency from the total diet consumed. The effect of sludge additions on weight gain is shown in Table 49. As in the Metro Denver sludge feeding trial, the sludge fed animals gained less weight and had a lower feed efficiency ratio than the control animals. The expected gains agreed with the observed gains when

TABLE 47. CONCENTRATION OF Zn, Cd, Cu, Hg, and Pb IN SLUDGE AND DIETS AMENDED WITH FT. COLLINS SEWAGE SLUDGE

Element	µg/g (Dry Weight)			
	Sludge		Ft. Collins Sludge Diets	
	Metro Denver	Ft. Collins	0%	12%
Zn	1500	1700	26.3	236
Cd	21	98	0.14	11.3
Cu	710	1700	7.7	213
Hg	11	18	<0.02	2.6
Pb	780	470	0.86	56.6

TABLE 48. AMOUNT OF FEED AND SLUDGE CONSUMED AND THE OVERALL PERCENT FEED EFFICIENCY OF CATTLE FED FT. COLLINS SLUDGE

Diet	(Kg) Total Weight Gain	Kg (Dry Weight) Dry Matter Consumed	Sludge Kg (Dry Wt) Consumed	% Feed Efficiency
0%	130	5,392	0	2.4
12%	120	5,694	652	2.1

TABLE 49. THE EFFECT OF FT. COLLINS SLUDGE IN DIETS UPON FEEDLOT CATTLE PERFORMANCE

Diet	Kg				Mean Daily Gain		
	Mean Body Weight After Days on Test			Mean Total Gain	Kg		Observed/ Expected
	0	63	106		Observed	Expected ¹	
0%	340	420	470a ²	130a	1.24	1.25	.99
12%	340	420	460 b	120 b	1.13	1.10	1.03

1. Expected gain calculated according to NRC (1970) from feed consumption assuming no energy contribution from sludge.
2. Values followed by different letters are significantly different at the 5% level (Student's t-test).

the sludge component of the diet was assumed to have no energy value and was removed when calculating expected gains. Results of Ft. Collins sludge on cattle performance were almost identical to results of the first feeding trial using Metro Denver sludge. The sludge was shown to have had no positive or negative effects on the general performance of the cattle other than to act as a diet diluent.

Postmortem histopathological findings are given in Appendix C. All animals remained healthy throughout the experiment and no signs of pathology related to the sludge component of the diet were evident.

Trace Element Concentration of Tissues From Beef Cattle Fed Ft. Collins Sewage Sludge - 3 Months Ingestion Period --

As in the previous studies the tissues that retained the highest concentrations of Cd, Cu, Pb, and Hg were the kidney and liver, while muscle tissues had the highest concentration of Zn. Table 50 shows the concentrations of Zn, Cd, Cu, Hg, and Pb in kidney and liver tissues. Zinc concentrations in liver and kidney tissues were not affected by sludge additions to the diet. Cadmium concentrations significantly increased in both kidney and liver tissues when Ft. Collins sludge was ingested. Kidney Cu concentrations decreased significantly from sludge ingestion; however, it is doubtful that this decrease was biologically significant.

The concentrations of Zn, Cd, Cu, Hg, and Pb in bone, lung, brain, spleen, and muscle tissues are shown in Table 51. Lead increased significantly in bone, brain and spleen tissues. Mercury increased in muscle, lung, and spleen tissues. Zinc and copper did not show any significant differences in bone, muscle, lung, brain or spleen tissues due to the ingestion of Ft. Collins sludge.

TABLE 50. MEAN CONCENTRATION OF Zn, Cd, Cu, Hg, AND Pb IN KIDNEY AND LIVER TISSUES OF BEEF CATTLE FED A DIET CONTAINING 0 AND 12% FT. COLLINS SLUDGE FOR 3 MONTHS

Tissue Type & % Sludge in Diet	$\mu\text{g/g}$ (Dry Weight)				
	Zn	Cd	Cu	Hg	Pb
Kidney - 0	93a ¹	1.2a	23a	0.09a	0.95a
Kidney - 12	96a	14. b	21 b	2.0 b	11. b
Liver - 0	142a	0.19a	127a	<0.01a	0.31a
Liver - 12	132a	4.9 b	113a	0.27 b	4.3 b

1. Values followed by different letters are significantly different at the 1% level, except for Cu which is significantly different at the 5% level (Student's t-test).

TABLE 51. MEAN CONCENTRATION OF Zn, Cd, Cu, Hg, AND Pb IN TISSUES OF BEEF CATTLE FED A DIET CONTAINING 0 AND 12% FT. COLLINS SEWAGE SLUDGE FOR 3 MONTHS

Tissue Type & % Sludge in Diet	$\mu\text{g/g}$ (Dry Weight)				
	Zn	Cd	Cu	Hg	Pb
Bone - 0	86 a ¹	<0.02a	0.37 a	<0.01a	5.0 a
Bone - 12	71 a	<0.01a	1.5 a	<0.01a	7.2 b
Muscle - 0	340 a	<0.01a	3.6 a	<0.01a	<0.01a
Muscle - 12	267 a	0.03a	3.2 a	0.02 b	<0.01a
Lung - 0	94.7a	0.02a	7.1 a	<0.01a	0.10a
Lung - 12	96.3a	0.48 b	7.1 a	0.05 b	0.23a
Brain - 0	32. a	<0.01a	5.2 a	<0.01a	<0.01a
Brain - 12	41. a	<0.01a	5.7 a	<0.01a	0.24 b
Spleen - 0	117. a	0.02a	4.4 a	<0.01a	0.21a
Spleen - 12	110. a	0.43 b	4.8 a	0.08 b	3.8 b

1. Values followed by different letters are significantly different at the 5% level (using Student's t-test).

As in the first feeding trial utilizing Metro Denver sludge there were significant increases of some heavy metals in tissues due to the ingestion of sewage sludge. None of the heavy metals were transferred to any significant extent to muscle tissues, demonstrating that the major edible portions of cattle were well protected from heavy metal accumulations.

Some Comparison Between the Ft. Collins and Metro Denver Feeding Trials in Relation to Heavy Metal Content of Cattle Tissues

Cadmium concentrations significantly increased in both renal and hepatic tissues when Ft. Collins sludge was ingested (Table 52). If the mean renal Cd concentrations of 14 ppm is compared with the renal Cd concentration of 2.4 ppm from the ingestion of an equivalent amount of Metro Denver sludge, a dose response can be seen; thus, a 5 fold increase in dietary Cd has led to an equivalent increase in renal Cd. When the two feeding trials are compared

TABLE 52. MEAN Cd, Zn, AND Cu TISSUE CONCENTRATIONS FROM CATTLE FED FT. COLLINS OR METRO DENVER SLUDGE

Tissue Type & % Sludge in Diet	$\mu\text{g/g}$ (Dry Weight)		
	Zn	Cd	Cu
Ft. Collins:			
Kidney - 0	93a ¹	1.2 a	23a
Kidney - 12	96a	14 b	21 b
Liver - 0	142a	0.19a	127a
Liver - 12	132a	4.9 b	113a
Metro Denver:			
Kidney - 0	84a	1.1 a	17a
Kidney - 12	82a	2.4 b	15 b
Liver - 0	87a	0.2 a	124a
Liver - 12	101 b	0.4 b	240 b

1. Means followed by a different letter within a column and tissue type are significantly different at the 5% level.

in relation to uptake of Cd into liver tissues, it can be seen that there was a 10 fold increase of hepatic Cd when Ft Collins sludge was ingested. Thus, the dose response of hepatic Cd was different than that of renal Cd. There was also a difference in dose response in relation to hepatic Cd when the two sludges are compared. The ingestion of Ft. Collins sludge led to a 25 fold increase in hepatic Cd; whereas, there was only a 2 fold increase from ingesting Metro Denver Sludge.

The differences in dose response in relation to Cd between the Ft. Collins and Metro Denver sludge feeding trials could be the result of a number of factors, a few of which are listed here. The fact that hepatic Cd increased 25 fold in the cattle ingesting Ft. Collins sludge could be the result of the higher Cd to Zn ratio found in the Ft. Collins sludge. The protective effect of a low Cd to Zn ratio has been shown by a number of investigators (Petering et al., 1971; Parizek, 1957; Supplee, 1963). Since Cu is known to affect Zn absorption, the high level of dietary Cu in the Ft. Collins feeding trial may be decreasing the amount of Zn being absorbed and in effect raising the Cd to Zn ratio. Both of these effects together would help explain the fact that Cd in the Ft. Collins sludge appeared to be more

TABLE 53. COPPER CONCENTRATIONS IN KIDNEY AND LIVER TISSUES OF BEEF CATTLE FED A DIET CONTAINING 0 AND 12% METRO DENVER OR FT. COLLINS SLUDGE

Tissue	% Metro Denver Sludge		μg/g (Dry Weight)	
	0	12	% Ft Collins Sludge 0	12
Kidney	17a ¹	15 b	23 c	21 d
Liver	124a	240 b	127a	113a

1. Values followed by different letters within a row are significantly different at the 5% level (Student's t-test).

available for uptake (particularly into liver tissues) than Cd from the Metro Denver sludge.

When data from the Metro Denver sludge feeding trial is examined it can be seen that there is a definite increase in hepatic Cu due to the ingestion of Metro Denver sludge (Table 53). However, there was no similar increase in hepatic Cu when Ft. Collins sludge was incorporated into the cattle diet, even though the Ft. Collins sludge diet contained more than twice as much Cu (89 ppm Cu versus 213 ppm Cu). When the mean hepatic Cu concentration from the Metro Denver sludge diet is compared with the hepatic Cu from the Ft. Collins sludge diet a very significant difference in Cu is apparent. The results shown here conflict with results of the long term (9 month) Ft. Collins sludge ingestion study, which has not been discussed as yet. However, analyses of liver biopsies from cattle ingesting Ft. Collins sewage sludge for 9 months indicated that Cu increased in a linear fashion throughout the sludge ingestion period.

Estimates of Beef Cattle Tissue Retention of Cd and Pb From the Ingestion of Sewage Sludge --

Body retention of dietary Cd in the form of salts has been estimated to be somewhere between 2 and 9% (Friberg et al., 1974; McLellan et al., 1978; Flanagan et al., 1978). Total body retention of Cd in this study was impossible to determine because tissues which make up as much as 50% of the body (muscle, bone, and fat) often contained levels of metals that were below our detection limits. Other tissues such as the mucosa of the alimentary canal were not examined and may have contained significant levels of metals; therefore, estimates of metal retention detailed in this report are not for the whole body and should not be construed as such, but rather they indicate trends and differences between the sludge diets.

Estimates of Cd retention from sewage sludge ingestion were made by estimating the average amount of increased retention in kidney and liver tissues of the cattle. Estimates of Pb retention were calculated by

estimating the increased retention in kidney, liver and bone tissues. Estimates of specific tissue weights were obtained from Moulton, et al. (1922).

Table 54 shows the total weight of feed consumed per animal, the concentration of Cd and Pb in the total diet, and the total amount of metal consumed per animal during the treatment periods. Table 55 shows the estimated amounts of Cd and Pb contained in kidney, liver, and bone tissue of cattle fed the sludge diets along with the consequent increases due to the ingestion of the two sewage sludges. If the increased metal retention is compared with the amount of Cd and Pb consumed during the treatment period a measure of (but not true) retention can be calculated. The retention of sludge Cd when the diet was composed of 12% Metro Denver sludge was 0.02%, while the calculated Cd retention for the Ft. Collins sludge was 0.07%. The differences in Cd retentions would indicate that the Cd contained in the Ft. Collins sludge was more available for absorption and retention into the cattle tissues than Cd from the Metro Denver sludge. While the retention values shown here are admittedly less than true retention values, it is doubtful that they are off by much more than one order of magnitude; thus, it would appear that the Cd contained in both sewage sludges was less available for absorption and retention into body tissues than that which has been shown in other animal species utilizing Cd salts (McLellan et al., 1978; Flanagan et al., 1978; Doyle et al., 1974).

Retention values for Pb were 0.85% for the Metro Denver sludge feeding trial and 0.23% for the Ft. Collins sludge feeding trial.

TABLE 54. MEAN WEIGHT OF DIET CONSUMED PER ANIMAL, THE CONCENTRATION OF Cd AND Pb IN THE TOTAL DIET, AND THE AMOUNT OF Cd AND Pb CONSUMED PER ANIMAL DURING THE TREATMENT PERIOD

<u>Treatment</u>	<u>Dry Matter Consumed</u>	<u>µg/g (Dry Wt.)</u>		<u>Total Metal Consumed (mg)</u>	
	<u>Kg</u>	<u>Cd</u>	<u>Pb</u>	<u>Cd</u>	<u>Pb</u>
Metro Denver Sludge Diet:					
0% Sludge	866	0.03	0.5	26	433
12% Sludge	870	2.4	80	2090	60,600
Ft. Collins Sludge Diet:					
0% Sludge	899	0.13	0.86	126	773
12% Sludge	949	11.3	56.6	10,720	53,000

TABLE 55. ESTIMATED AMOUNT OF Cd AND Pb CONTAINED IN KIDNEY, LIVER, AND BONE TISSUE OF CATTLE FED DIETS OF 0 OR 12% SEWAGE SLUDGE FOR 3 MONTHS

Element	Metro Denver Sludge Diet			Ft. Collins Sludge Diet		
	% Sludge	Increase Due		% Sludge	Increase Due	
	0	12	To Sludge	0	12	To Sludge
	mg (Dry Weight)					
Cd	0.43	0.82	0.39	0.44	8.4	7.9
Pb	44	633	589	285	407	122

Refractory Organic Residues In Tissue of Cattle Fed Ft. Collins Sewage Sludge for 3 Months --

The levels of chlorinated hydrocarbon residues determined in the sludge, feed, and silage are shown in Table 56. The concentrations of the various compounds are very similar to the levels found in the first feeding trial utilizing Metro Denver sewage sludge (Table 45). The Ft. Collins sludge

TABLE 56. MEAN CONCENTRATION (ppb, WET WEIGHT BASIS) OF REFRACTORY ORGANICS DETERMINED IN SLUDGE, FEED AND SILAGE¹

Compound	Sludge ²	Silage ³	Feed ⁴	
			0% Sludge	12% Sludge
p,p'-DDE	121	<1.0	<10.0	31
alpha-BHC	<4.0	<1.0	<10.0	<10.0
beta-BHC	<19.0	ND ⁵	ND	<10.0
Dieldrin	39	ND	<10.0	<10.0
Oxychlordane	28	ND	ND	<10.0
HCB	<5.7	<1.0	ND	<10.0
Chlordane	1047	ND	ND	188
AR-1254	4434	<40.0	<500	758

1. Mean of 6 separate composite samples.
2. % moisture ~5%.
3. % moisture ~67%.
4. % moisture ~10%.
5. ND - Not Detected.

contained slightly higher concentrations of p,p'-DDE and AR-1254, while the Metro Denver sludge contained slightly higher levels of oxychlordane, and dieldrin. However, for the most part, the two sludges were comparable, as were the sludge feed mixtures that were fed to the cattle during the two experiments. Trace concentrations of DDE, alpha-BHC, HCB, and AR-1254 were found in the silage which differed from the first feeding trial, where AR-1254 was the only refractory organic residue detected. No residues were detected in the water.

Concentrations of refractory organics were not determined in muscle tissue because the levels found in the first feeding trial with Metro Denver sludge did not increase due to the ingestion of sludge. For the same reasons, samples of brain and blood were not analyzed for persistent organic residues.

Residues that were detected in liver and abdominal fat tissues are shown in Tables 57 and 58. Traces of beta-BHC, dieldrin, oxychlordane, and AR-1254 were detected in liver tissues as a result of adding sludge to the cattle diet.

Abdominal fat tissues (Table 58) showed significant increases in a few residues due to the ingestion of sludge. Trace levels of p,p'-DDE, dieldrin, heptachlor epoxide, oxychlordane, and TNC were detected in the fat of control animals. Apparent increases in p,p'-DDE, alpha-BHC, dieldrin, oxychlordane, HCB, and AR-1254 resulted from sludge additions to the diet.

All of these persistent chlorinated hydrocarbons are below the tolerance levels that have been set by FDA for fish or other food products. The compound that accumulated to the highest concentration in both feeding trials was AR-1254, which at 1672 ppb was below the tolerance level of 3 ppm that has been set for red meat animals (Food and Drug Administration, 1981).

The studies just discussed were conducted with young animals that were gaining weight rapidly; therefore, it can be assumed that at least a portion of the absorbed chlorinated hydrocarbons were effectively diluted by fat. It is possible that if these animals had been older and not gaining weight, the concentrations of the various chlorinated compounds could have been higher.

NINE MONTH INGESTION PERIOD

Young animals are known to absorb greater percentages of dietary heavy metals (particularly Pb) than older animals, but the extent to which this might occur in cattle has not been investigated. In the studies previously reported, feeding trials were conducted utilizing young animals; thus, the uptake rates may have been higher than would have occurred with older animals. In a typical cow-calf operation where sludge would be added to pasture land, the cows would be exposed to sewage sludge for a number of years, while the younger cattle would be sold off. Thus, the greatest exposure to sewage sludge would be to those breeding animals that are kept for a number of years.

TABLE 57. MEAN CONCENTRATION OF CHLORINATED HYDROCARBONS WITHIN LIVER TISSUES (ppb WET WEIGHT BASIS)¹.

Compound	Diet	
	Control	12% of Sludge
p,p'-DDE	< 10	< 10
alpha-BHC	ND ² .	ND
beta-BHC	ND	< 10
Dieldrin	ND	< 10
Oxychlordane	ND	< 10
AR-1254	ND	< 500

1. % moisture of liver ~67% with mean of 1.8% fat.

2. ND - Not Detected.

TABLE 58. MEAN CONCENTRATION OF CHLORINATED HYDROCARBONS IN ABDOMINAL FAT OF BEEF CATTLE FED SEWAGE SLUDGE (ppb WET WEIGHT)¹.

Compound	Diet	
	Control	12% of Sludge
p,p'-DDE	< 10	74
alpha-BHC	ND ² .	< 10
beta-BHC	ND	ND
Dieldrin	< 10	30
Heptachlor Epoxide	< 10	< 10
Oxychlordane	< 10	18
TNC	< 10	< 10
Hexachlorobenzene	ND	< 10
Chlordane	ND	ND
AR-1254	ND	1672

1. % moisture ~11% with mean fat ~84%.

2. ND - Not Detected.

Another aspect which was not examined in the previous studies was the possibility that the cattle could eliminate some portion of the sludge-born heavy metals or persistent organics after being removed from the sludge diet. The U.S.D.A. had also reported severe joint deterioration in cattle from grazing pastures after surface applications of liquid sludge (Anonymous, 1977). Subsequent work showed that the joint deterioration they observed was probably related to the high iron sludge that was being used in their grazing studies. But, because of their original findings, we were interested in health effects to cattle from long term exposure to high levels of sewage sludge.

Two groups of cattle (16 each) were selected for this study; one group of older Hereford cows ranging in age from 4 to 7 years, and a younger group of 5 month old Hereford steers. The cows were lotted into two uniform groups of 8 on the basis of weight and age, so that each group had an average age of 5. Steers were lotted into two groups of 8 according to weight. One group of cows and steers were placed on a control diet and one group of cows and steers were fed a diet containing approximately 12% (dry weight) Ft. Collins anaerobically digested sludge for a period of 9 months. The cow diets were fed at maintenance levels, while the steer diet was fed and adjusted to maintain a 0.8 kg per head per day live weight gain. The amounts of diet components consumed by the cattle during the 9 month sludge feeding period are shown in table 59. The estimated concentration of trace metals contained in the cattle diets are summarized in table 60. The elemental composition of the various diets were calculated by summing the amount of metals contained in each diet component that was consumed by each treatment group (table 59) and dividing by the total dry weight of those diet components.

Performance of Cattle Fed Ft. Collins Sewage Sludge for 9 Months

Sludge fed and control cows showed a positive weight gain during the 9 month sludge ingestion period (Table 61); however, the control cows gained slightly more weight with an average weight gain of 0.29 kg/day versus 0.17

TABLE 59. AMOUNT OF DIET COMPONENTS CONSUMED BY CATTLE DURING 9 MONTH SLUDGE FEEDING PERIOD

	kg (Dry Weight)			
	Cows		Steers	
	0%	12%	0%	12%
Silage	9,689	9,392	10,530	10,205
Alfalfa	1,142	1,144	1,398	1,170
Protein Supplement	0	0	880	886
Sludge	0	1,571	0	1,690

TABLE 60. ELEMENTAL CONCENTRATION OF SLUDGE AND DIETS FED (CALCULATED) TO CATTLE DURING 9 MONTH SLUDGE INGESTION PERIOD

Element	$\mu\text{g/g}$ (Dry Weight)				
	Sludge ¹	Cow Diet ²		Steer Diet ²	
		0%	12%	0%	12%
Cd	88	0.1	11.5	0.1	10.7
Zn	1617	22	230	32	220
Pb	387	1.8	50	1.8	50
Cu	1356	4.4	180	6.0	170
Fe	11800	340	1830	360	1760
Ca	15670	260	2260	326	2190
P	16270	1740	3620	2800	4490

1. Mean of 7 composite samples. Sludge composite samples were collected at each batch mixing.
2. From 4 composite silage samples, 4 composite alfalfa samples, and 4 composite supplement samples.

TABLE 61. MEAN COW AND STEER WEIGHT DURING FT. COLLINS SLUDGE FEEDING TRIAL

Days on Experiment	kg			
	Cows		Steers	
	% OF SLUDGE IN DIET 0%	12%	% OF SLUDGE IN DIET 0%	12%
0	504	488	224	216
90	520	503	323	298
180	532	503	358	342
270	581	535	446	418
312 ¹	566	547	414	401
354	552	542	442	420
397 ²	541	517	447	424

1. First weighing after sludge was removed from diet, average of 4 animals.
2. Shrunk weights - weight taken prior to sacrifice, animals off feed and water for 16 hours.

kg/day for the sludge fed cows. The sludge fed and control steers also showed a positive weight gain over the 9-month sludge ingestion period (Table 61). The control steers gained 0.82 kg/day while the sludge fed steers gained 0.75 kg/day. General performance of the cattle was similar to results obtained from the two previously described sludge feeding trials. The addition of sludge to the cattle diets appeared to have no positive or negative effects on cattle performance, other than to act as a diet diluent. All animals, except one, remained healthy throughout the experiment, and no signs of pathology related to the sludge component of the diet was evident. Inspection of bone joints at the time of slaughter revealed no joint deterioration. A detailed report describing the antemortem inspection, necropsy, and histopathologic examination of tissues and organs from the cattle is included in Appendix C.

Trace Element Concentration of Beef Cattle Tissues Fed Ft. Collins Sewage Sludge - 9 Month Ingestion Period

At the conclusion of the 9 month sludge feeding period, 4 animals from each group were slaughtered, and samples of kidney, liver, muscle, and bone tissues taken for heavy metals analyses. The remaining animals were kept on the test for another 4 months, but sludge was removed from the diet of treatment animals. The remaining 16 animals were slaughtered and sampled at the end of the 4 month withdrawal period.

Table 62 summarizes the concentration of heavy metals determined in kidney tissues of the cows and steers after 9 months of sludge ingestion and 4 month withdrawal period. There were no significant differences in metal retention levels between the cows and young steers. Concentrations of Cd, Pb and Zn increased significantly, while Fe and Cu concentrations were not affected by the sludge ingestion. Kidney Cd and Zn increased during the sludge ingestion period and appeared to continue increasing during the withdrawal period. The steers displayed an average increase of kidney Zn and Cd during the 13 month study that was equimolar. This effect has been noted by Schroeder et al. (1967); Anke et al. (1970), and Elinder et al. (1977), but was not observed in any of the sludge feeding or "age" studies previously discussed.

Liver Cd, Pb, and Cu increased significantly due to the sludge ingestion (Table 63). Copper and Pb significantly decreased during the sludge withdrawal period, while Cd remained unchanged. The cows showed a significant increase in liver Zn after being withdrawn from the sludge; however, it is doubtful that this was a sludge effect. The retention levels and depletion rates of Pb and Cu were not significantly different between the young steers and older cows.

Analysis of liver biopsies showed that Cd increased in liver tissues with time in a linear fashion (Fig.4). No upper limit or "Cd saturation point" was reached during the 9 month sludge ingestion period. Cadmium did not decrease significantly during the withdrawal period, and there were no differences in rates of uptake or retention between the young steers and older cows.

TABLE 62. HEAVY METALS CONCENTRATIONS IN KIDNEY TISSUES OF CATTLE AFTER 9 MONTHS OF SLUDGE INGESTION AND A 4 MONTH WITHDRAWAL PERIOD

Treatment	$\mu\text{g/g}$ (Dry Weight)				
	Cd	Pb	Zn	Fe	Cu
Cows:					
Control ¹	7.4a ⁴	1.4a	77a	320a	17a
Sludge Fed ²	54.0 b	4.3 b	88 b	281a	15a
Sludge Withdrawn ³	69.0 b	3.4 b	131 c	343a	17a
Steers:					
Control ¹	3.5a	1.1a	82a	255a	19a
Sludge Fed ²	57.0 b	5.2 b	98 b	290a	16a
Sludge Withdrawn ³	64.0 b	3.4 b	116 c	247a	16a

1. Average of all control animals, 9 month and 4 month withdrawal period.
2. Cattle fed diet containing 12% sludge and slaughtered at 9 months.
3. Cattle fed diet containing 12% sludge and slaughtered after 4 month withdrawal period.
4. Values within a group followed by different letters are significantly different at the 5% level using Student's t-test.

TABLE 63. HEAVY METALS CONCENTRATIONS IN LIVER TISSUES OF CATTLE AFTER 9 MONTHS OF SLUDGE INGESTION AND A 4 MONTH WITHDRAWAL PERIOD

Treatment	$\mu\text{g/g}$ (Dry Weight)				
	Cd	Pb	Zn	Fe	Cu
Cows:					
Control ¹	1.22a ⁴	.65a	108a	434a	62a
Sludge Fed ²	19.0 b	4.9 b	118a	525a	212 b
Sludge Withdrawn ³	21.6 b	1.2 c	141 b	596a	118 c
Steers:					
Control ¹	0.91a	<0.5a	128a	228a	32a
Sludge Fed ²	19.9 b	4.1 b	138a	330a	393 b
Sludge Withdrawn ³	17.5 b	1.5 c	130a	234a	126 c

1. Average of all control animals, 9 month and 4 month withdrawal period.
2. Cattle fed diet containing 12% sludge and slaughtered at 9 months.
3. Cattle fed diet containing 12% sludge and slaughtered after 4 month withdrawal period.
4. Values within a group followed by different letters are significantly different at the 5% level using Student's t-test.

Copper increased significantly in liver tissues during the sludge ingestion period (Fig.5). The rate of Cu uptake or retention into liver tissues also appeared to be linear. The steers showed a slightly higher uptake rate or retention level than the cows, but this difference was not statistically significant. Both groups of cattle showed a significant decrease in liver Cu concentrations during the sludge withdrawal period. The concentration of Cu in liver tissues of all control cattle decreased slightly during the time period of the study; however, this decrease was not statistically significant.

Lead concentrations in liver tissues increased significantly during the sludge ingestion period and decreased to near normal or control levels by the end of the withdrawal period (Fig. 6).

Concentrations of Fe and Zn in the liver biopsies were not significantly affected during or following the sludge ingestion period.

Concentrations of Pb in the bone biopsies increased significantly during the first 3 months of the sludge ingestion period, but did not increase significantly after that time period (Fig.7). There was no decrease in bone Pb after sludge was withdrawn from the diet. The older control cows had significantly higher concentrations of Pb in bone, (mean of 2 ppm) than the younger control steers which had an average concentration of 1 ppm (Fig.7). Other trace metals (Zn, Cd, Cu, and Fe) were not affected by the sludge ingestion.

Iron concentrations of the tail bone biopsies averaged 174 ± 103 ppm (dry weight), which was considerably higher than bone samples (tarsal bone) taken at slaughter, which averaged 20 ± 1 ppm. The large difference in Fe concentration between the two bone types was probably related to the fact that the vertebrae contained large volumes of porous bone containing blood. Other trace metals (Pb, Zn, Cu, and Cd) were found in the same concentrations in both bone biopsy and slaughter samples. Concentrations of trace metals found in the tarsal bone samples, taken at slaughter, are shown in Table 64. Lead concentrations were again seen to significantly increase due to the sludge ingestion. Other trace metals were not affected.

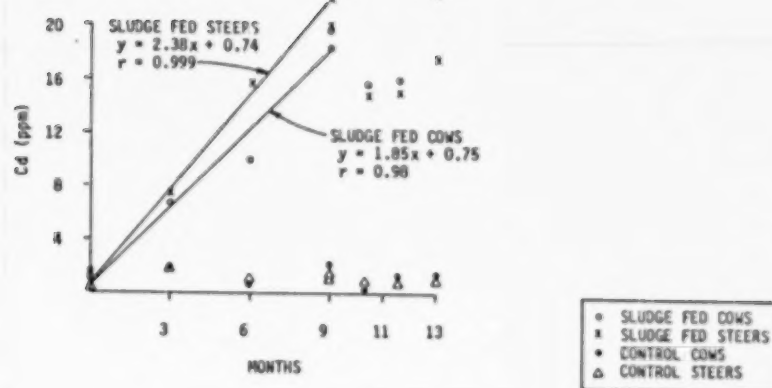


Figure 4. Cadmium concentrations (dry weight) of liver biopsies during 9 month sludge ingestion period and 4 month withdrawal period.

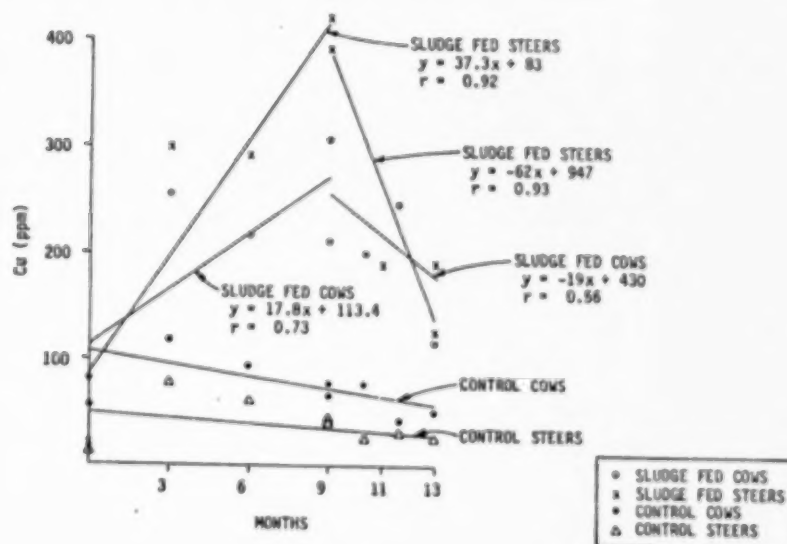


Figure 5. Copper concentrations (dry weight) of liver biopsies during 9 month sludge ingestion period and 4 month withdrawal period.

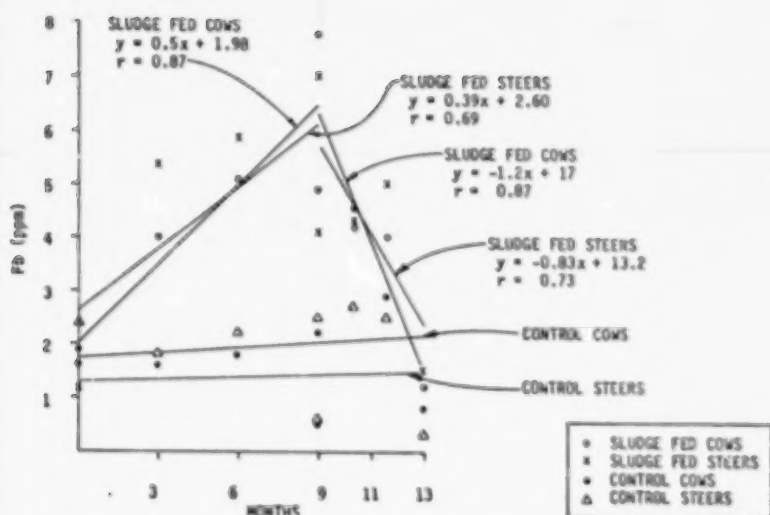


Figure 6. Lead concentrations (dry weight) of liver biopsies during 9 month sludge ingestion period and 4 month withdrawal period.

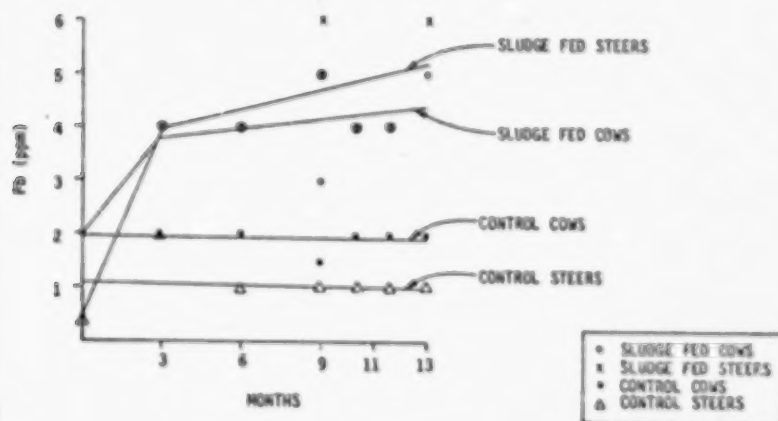


Figure 7. Lead concentrations (dry weight) of bone biopsies during 9 month sludge ingestion period and 4 month withdrawal period.

TABLE 64. HEAVY METALS CONCENTRATIONS IN TARSAL BONE TISSUES OF CATTLE AFTER 9 MONTHS OF SLUDGE INGESTION AND A 4 MONTH WITHDRAWAL PERIOD

Treatment	$\mu\text{g/g}$ (Dry Weight)				
	Pb	Cd	Zn	Cu	Fe
Cows:					
Control ¹	2a	<0.05	67	2	20
Sludge Fed ²	3 b	<0.05	64	2	21
Sludge Withdrawn ³	5 b	<0.05	67	2	22
Steers:					
Control ¹	1a	<0.05	66	2	19
Sludge Fed ²	6 b	<0.05	58	2	19
Sludge Withdrawn ³	6 b	<0.05	54	2	19

1. Average of all control animals, 9 month and 4 month withdrawal period.
2. Cattle fed 12% sludge diet and slaughtered at 9 months.
3. Cattle fed 12% sludge diet and slaughtered after 4 month withdrawal period.
4. Values within a group followed by different letters are significantly different at the 1% level using Student's t-test.

TABLE 65. HEAVY METALS CONCENTRATIONS IN MUSCLE TISSUES OF CATTLE AFTER 9 MONTHS OF SLUDGE INGESTION AND A 4 MONTH WITHDRAWAL PERIOD

Treatment	$\mu\text{g/g}$ (Dry Weight)				
	Cd	Pb	Zn	Cu	Fe
Cows:					
Control ¹	0.12a ⁴	<0.4	280	100	3.7
Sludge Fed ²	0.27 b	<0.2	265	98	3.9
Sludge Withdrawn ³	0.21 b	<0.4	286	92	2.9
Steers:					
Control ¹	0.14a	<0.4	293	86	4.4
Sludge Fed ²	0.43 b	<0.5	280	89	5.5
Sludge Withdrawn ³	0.15a	<0.3	297	81	3.2

1. Average of all control animals, 9 month and 4 month withdrawal period.
2. Cattle fed 12% sludge diet and slaughtered at 9 months.
3. Cattle fed 12% sludge diet and slaughtered after 4 month withdrawal period.
4. Values within a group followed by different letters are significantly different at the 5% level using Student's t-test.

Table 65 summarizes the heavy metals concentrations found in muscle tissues. From the data, it appears that Cd increased significantly in muscle tissues during the sludge ingestion period. These results are surprising since other investigators have fed higher levels of dietary Cd which did not result in elevated levels of Cd in muscle tissues. For example, Osuna et al. (1979) did not find increased Cd in muscles of swine when diets containing 83 ppm Cd (as CdCl₂ or Chicago sewage sludge) were fed. Because of the unusually high muscle Cd levels found in even the control muscle tissues, the possibility of contamination should not be ruled out.

The Cd concentration in muscle tissues of cows was not affected when sludge was removed from the diet during the withdrawal period; however, it appears that Cd levels in muscle tissues of steers decreased significantly during the withdrawal period. The level of this decrease could not be accounted for by taking into account the amount of weight gain or dilution effect of increased tissue content of the growing steers. Other metals (Pb, Zn, Fe, and Cu) in muscle tissues were not affected by the sludge ingestion.

Refractory Organic Residues In Fat Tissues Of Cattle Fed Ft. Collins Sewage Sludge For 9 Months

Samples of sewage sludge, silage, hay, dietary supplement and fat tissues were analyzed for the refractory organics shown in Table 6. The average concentration of organic residues that were determined in feed components is shown in Table 66; residues not shown were not detected. Residues detected in the omental fat tissues of the cattle are shown in Table 67. Those residues not shown were not detected. DDE and TNC increased significantly in the fat tissues due to the sludge ingestion, but decreased significantly during the 4 month withdrawal period; indicating that both compounds were either metabolized or eliminated from the fat tissue. Dieldrin, PCB, HCB, and oxychlordane all showed significant increases due to the sludge ingestion, and decreased after the withdrawal period; however, the decreases were not statistically significant. Trace levels of DDT, heptachlor epoxide, and chlordane were found in most of the fat samples analyzed, but were not elevated due to the sludge ingestion.

PCB's in sewage sludges are usually found in higher concentrations (see Table 1) than other halogenated hydrocarbons; therefore, PCB's represent a greater potential hazard to grazing livestock and the human food chain than other halogenated hydrocarbons. The U.S. Food and Drug Administration has established lower PCB tolerance levels in milk fat than body fat; thus, most PCB studies relating to tissue and product residues have used dairy cattle. A number of studies have shown that the maximum PCB concentration reached in milk fat is 4 to 5 times that of the dietary exposure (Fries et al., 1973; Willett, 1975). Residue levels found in body fat were always less than that found in the milk fat, but this may be a result of the fact that a steady state level in milk fat is reached in a manner of weeks, compared to months for body fat. Long term studies examining body fat levels of PCB's in non-lactating animals have not been conducted; however, PCB's would be expected to behave like other halogenated hydrocarbons (Fries et al., 1972;

TABLE 66. MEAN CONCENTRATION OF ORGANIC RESIDUES DETERMINED IN FEED COMPONENTS FROM 9 MONTH SLUDGE INGESTION STUDY

Compound	ppb (Wet Weight)			
	Sludge ¹	Hay	Silage	Dietary Supplement
O, P' - DDT	ND ²	T	ND	ND
P, P' - DDT	T ³	T	ND	ND
P, P' - DDD	T	T	T	T
P, P' - DDE	148	T	ND	ND
Dieldrin	49.8	T	ND	ND
Heptachlor Epoxide	ND	T	ND	ND
PCB	6080	T	T	T
Oxychlordan	10	ND	T	ND
HCB	9.2	T	T	ND
Chlordane	1200	ND	T	ND
Lindane	ND	ND	T	ND
Heptachlor	ND	ND	T	ND

1. Mean % moisture of sludge was 75.
2. ND - Not detected
3. T - Trace, below practical detection limits. See Appendix A for detection limits.

TABLE 67. AVERAGE CONCENTRATION OF ORGANIC RESIDUES DETERMINED IN FAT TISSUES OF CATTLE FED FT. COLLINS SLUDGE

Compound	ppb (Wet Weight) ¹					
	Cows			Steers		
	Control ²	9 Mos ³	13 Mos ⁴	Control	9 Mos	13 Mos
P, P' - DDT	T ⁵	T	ND ⁶	T	T	T
P, P' - DDE	<12 a ⁷	259 b	135 c	50 a	242 b	122.4 c
Dieldrin	T a	35.8 b	24.6 b	T a	44.2b	28.7 b
Heptachlor Epoxide	T	T	T	T	T	T
PCB	T a	4224 b	3787 b	T a	4256 b	3758 b
Chlordane	T	T	T	T	T	T
TNC	T a	< 18 b	T a	T a	13.9b	T a
HCB	T a	< 13 b	12.3 b	T a	13.1b	T a
Oxychlordan	T a	17.8 b	<14.8 b	T a	20.8 b	16.7 b

1. Mean % fat content for all samples was 73.
2. Average of all control animals, 9 month and 4 month withdrawal period.
3. Cattle fed diet of 12% sludge and slaughtered at 9 months.
4. Cattle fed diet of 12% sludge and slaughtered after 4 month withdrawal period.
5. T - Trace, below practical detection limits. See Appendix A for detection limits.
6. ND - Not detected.
7. Values within a row followed by different letters are significantly different at the 5% level using Student's t-test.

Fries, 1977) and thus can be compared to those compounds. Body fat residues (heptachlor, dieldrin, DDE, and DDT) in steers and sheep have generally shown that the maximum concentrations reached in fat tissues are 5 to 6 times the dietary concentration (Bovard et al., 1971; Davison, 1970; Harrison et al., 1970).

The sludge feeding studies described here showed that the concentration factor from the dietary level to the body fat level was approximately 6, which agrees well with previous work utilizing other compounds. The two previous 3 month sludge ingestion studies (Tables 45 and 56) showed concentration factors of less than 2 (Metro Denver sludge) and about 3 (Ft. Collins sludge) indicating that the maximum or steady state body fat level was probably not reached within a 3 month period. Fries (1977) showed that the half life of AR-1254 and DDE in lactating dairy cows was approximately 40 to 70 days. Results from this study (Table 67) would indicate that the half life of DDE in non-lactating cattle would be approximately 120 days, and considerably longer for PCB's.

SECTION 7

PATHOGEN SURVIVAL DURING AIR DRYING OF SLUDGES

Anaerobically digested liquid primary sewage sludge was added to an earthen drying basin on January 13, 1977. Sampling was conducted at 3 week intervals until July 11, 1977. Plots of the microbiological and moisture data are shown in Figures 8 and 9. The weather data shown was extracted from the National Oceanic and Atmospheric Administration's 1976 Report for Greeley, Colorado. The rate of inactivation of the indicator virus gradually increased until March 15 (9 weeks). At this time there was a sharp increase in the rate of inactivation, probably as a result of both drying of the sludge and higher ambient temperatures. By the 3rd of May (15 weeks) f2 bacteriophage had decreased to only 3 PFU/g (plaque forming units per gram). Extrapolation of the data indicate that about 16 weeks was required for a seven log reduction of f2.

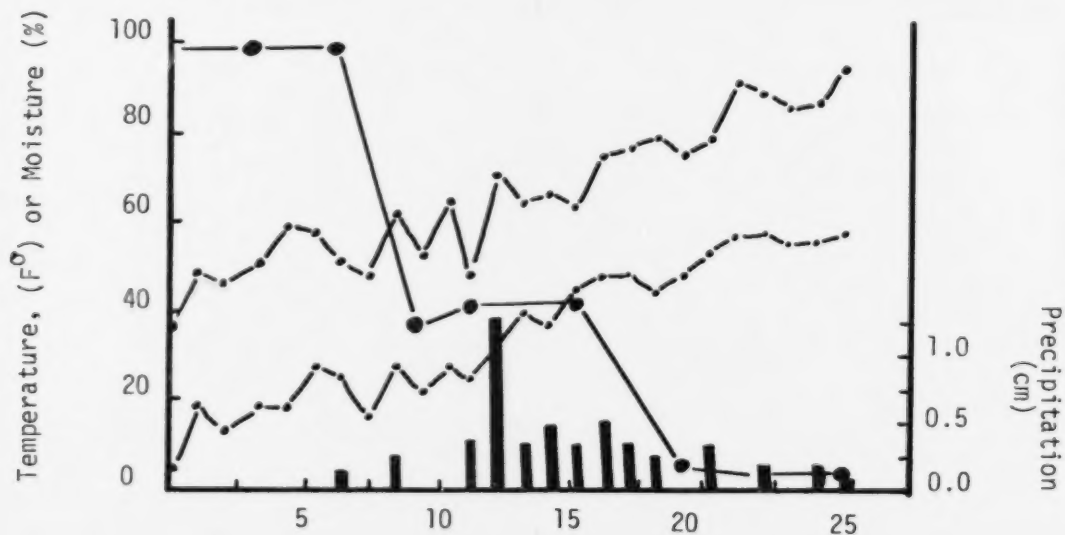


Figure 8. Sludge moisture content (O), average weekly high and low ambient temperatures (.), and precipitation (bars).

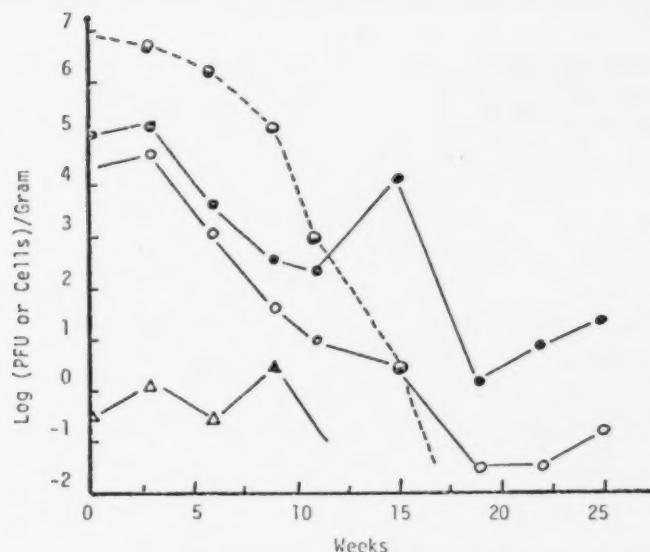


Figure 9. Survival of f2 indicator virus ○ total coliforms ●, fecal coliforms ○, and salmonellae △ (▲less than) in anaerobically digested sludge in drying basin 1 for the period of 13 January to 11 July 1977.

Salmonellae were found at low levels from the beginning of the study and were not detected after March 23rd (10 weeks). There was a 6 fold reduction in the fecal coliform population by May 10 (18 weeks), but total coliforms were still present in the last sampling of July 11 (25 weeks). None of the samples of soil water showed a positive test for any of the organisms examined.

The second basin (experiment 2) had sludge added to it on April 20, 1977. The inactivation of f2 phage was much more rapid than in the previous study, with a seven log reduction occurring in nine weeks. In this study (Figures 10 and 11) salmonellae were detected initially, but were not detected again until the month of October. Although the sludge showed a positive test for salmonellae, their numbers were too low to obtain MPN (most probable number) counts on October 10 (27 weeks). They grew to countable levels by the next sampling and remained detectable until the 67th week. Fecal coliforms were detectable up to the 73rd week. The reason for the excessive survival of salmonellae and coliforms in this experiment is not known, but reinfection from an outside source should not be ruled out. Three salmonellae serotypes: Braederup, Stanley, and Muenster were identified in the sludge. No attempt was made to identify serotypes in the first or subsequent experiments. After 60 weeks the moisture level was probably the factor that caused the eventual disappearance of salmonellae and fecal coliforms.

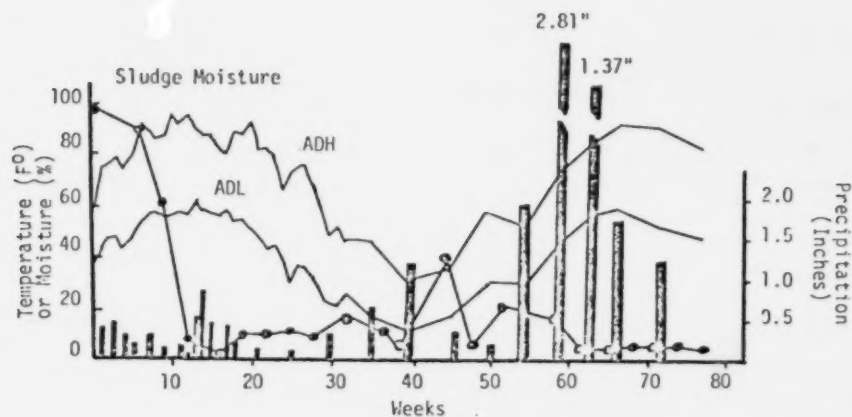


Figure 10. Experiment 2 - Sludge moisture ●, average daily high (ADH) and average daily low (ADL) temperatures and precipitation (bars). Up to week 30 ADH, ADL, and precipitations are shown on weekly basis, after week 30 they are shown on monthly basis.

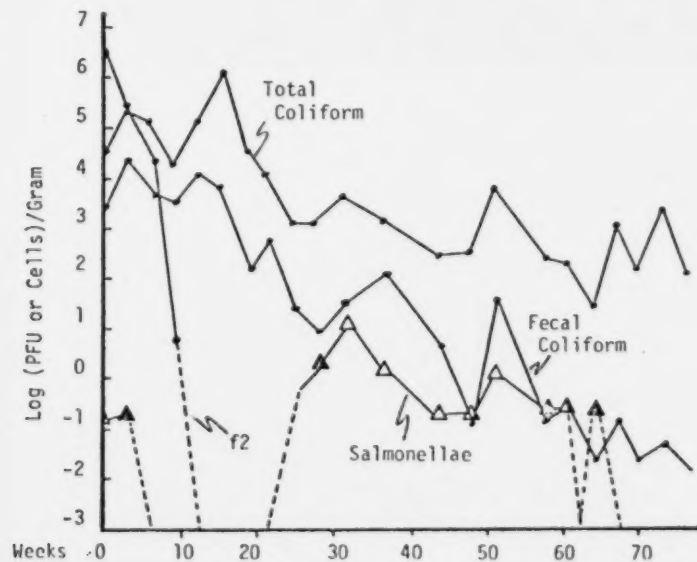


Figure 11. Survival of f2 indicator virus, total coliforms, fecal coliforms, and salmonellae Δ (Δ =less than) in anaerobically digested sludge for the period of April 20, 1977 to October 8, 1978.

Experiment 3 was initiated on July 15, 1977, and the collected data is shown in Figures 12 and 13. The sludge for this study was placed on top of the sludge from the first study which had dried to a thin layer approximately 1 cm in depth. About 14 weeks were required for destruction of seven logs of

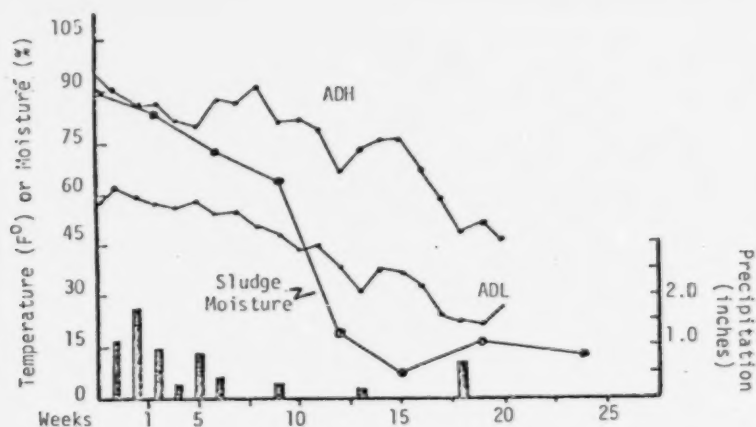


Figure 12. Sludge moisture, average daily high (ADH) and low (ADL) ambient temperatures, and precipitation (bars) for the period July 15, 1977 to January 5, 1978.

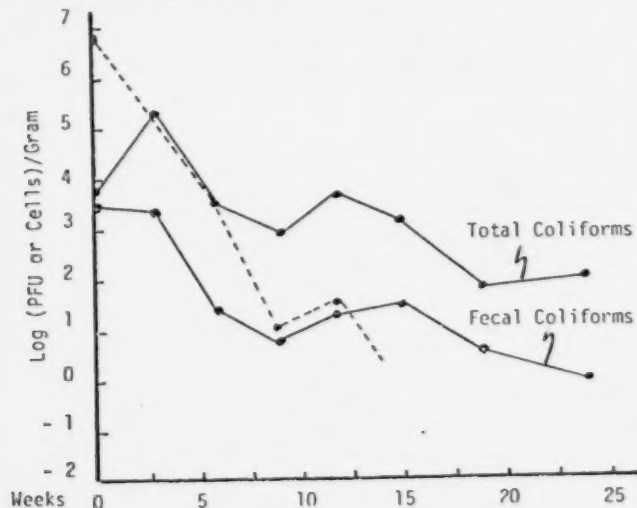


Figure 13. Survival of f2 indicator virus, total coliforms, and fecal coliform in anaerobically digested sludge for the period of July 15 to January 5, 1978.

f2 during the late summer and fall period. The sludge did not contain salmonellae in sufficient numbers to count, but tests were positive through the 15th week, after which they were negative. Fecal coliforms were no longer detectable by the 27th week of the experiment.

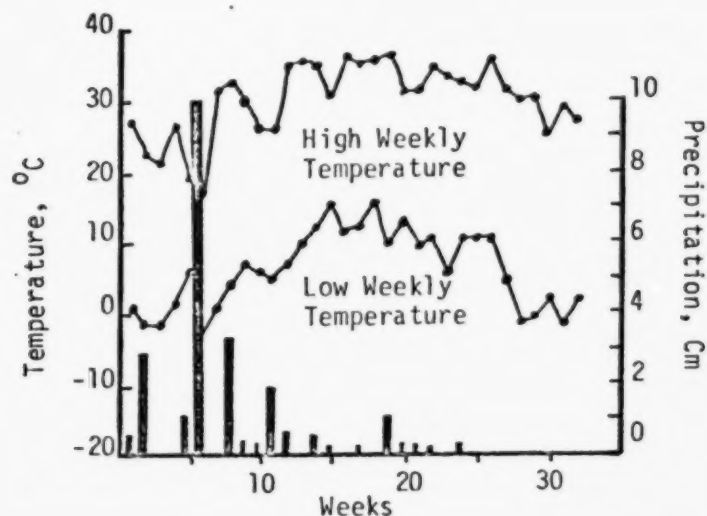


Figure 14. Average weekly high and low ambient temperatures and precipitation (bars) for Experiment 4.

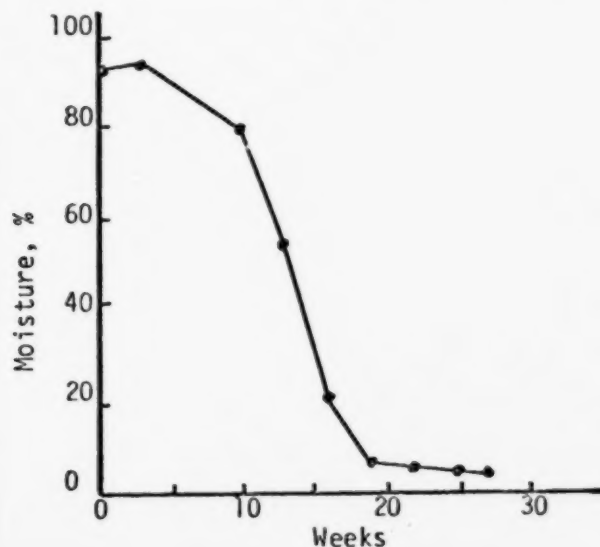


Figure 15. The moisture content of anaerobically digested sludge of Experiment 4, March 29 to August 19, 1978.

The fourth experiment was initiated on March 29, 1978. Precipitation, ambient temperatures, and sludge moisture content are shown in Figures 14 and 15. Data shown in Figure 16 appears to show that the f2 bacteriophage was inactivated at a higher rate than T7, but a larger quantity of f2 was initially present. Neither bacteriophage was detectable by the 19th week. Fecal coliforms were undetectable by the 19th week, but appeared to regrow and then dropped to undetectable levels by the 27th week (Figure 17). Salmonellae persisted for 14 weeks and were not detected after that.

The increased rate of inactivation of T7 and f2 was most likely a result of the increasing ambient temperature and the decreasing sludge moisture as shown in Figures 12 and 13. Since the higher temperatures might actually favor growth of salmonellae and fecal coliform their decrease is most likely related to the decreasing moisture content of the sludge.

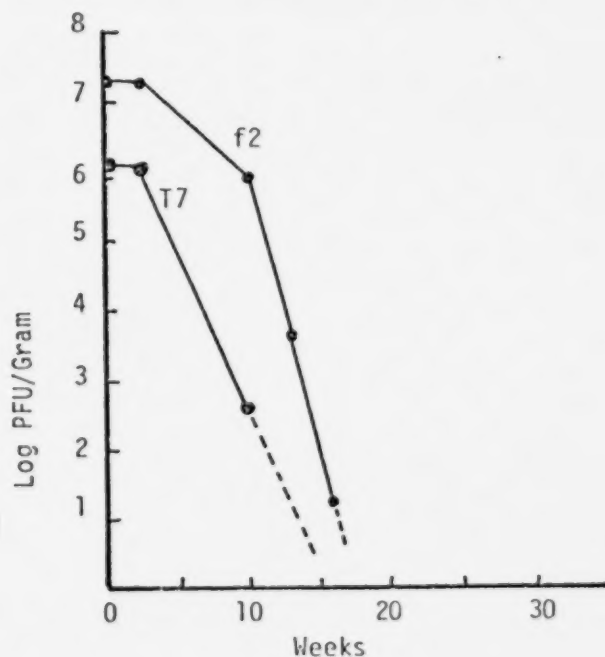


Figure 16. Survival of bacteriophages (f2 and T7) in drying sludge of Experiment 4.

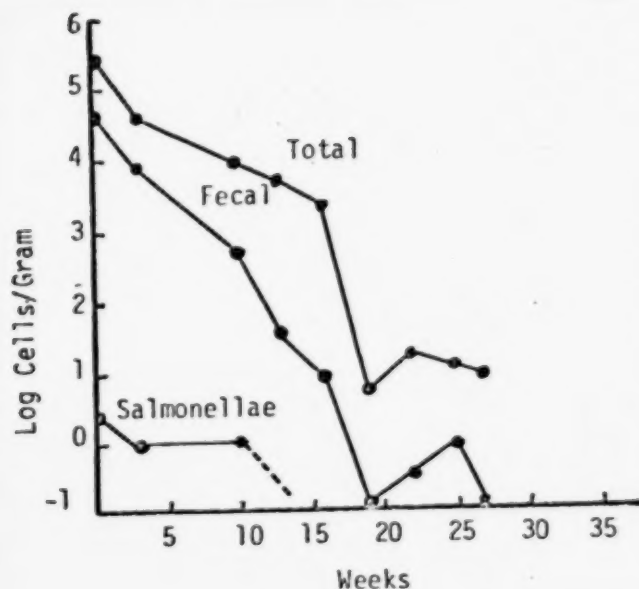


Figure 17. Survival of total and fecal coliforms and salmonellae in drying sludge of Experiment 4.

The results of these studies show that low temperatures favor the survival of bacterial and viral pathogens in the sewage sludge placed in the drying basins, but even at the low temperatures encountered in the winter months of January and February, inactivation of the indicator virus f2 was from 12 to 19 weeks. Because f2 is noted for its resistance to destruction by drying this would seem to set a maximum survival time under these conditions for enteroviruses. However, since there is no actual data comparing f2 survival in sludge with enteroviruses, this statement must be considered conjecture.

The result of the bacterial data (Table 68) show that both fecal coliforms and salmonellae can regrow in the sludge of the drying basins. However, other enteric pathogenic bacteria with the exception of shigella bacteria should not. Since shigella are not generally as hardy as salmonellae, the absence of salmonellae should indicate their absence also. The destruction of fecal coliforms should indicate the lack of survival of other pathogenic bacteria; however, other pathogenic bacteria will have to be studied before this can be said with absolute certainty.

TABLE 68. LENGTH OF SURVIVAL OF f2, FECAL COLIFORMS, AND SALMONELLAE IN SLUDGE DRYING BEDS

Experiment	f2	Weeks of Survival	
		Fecal Coliforms	Salmonellae
1	17	17	11
2	12	73	70
3	15	27	15
4	19	27	14

The analysis for ascaris ova in the ova seeded sludge of Experiment 4 showed a decrease in numbers and viability. Viability was determined by the Nile blue test of ova recovered as the sludge dried (Table 69). There was little change in infectivity between 91.8 and 20.3% moisture, but upon dropping to 7.7% moisture, a large decrease occurred.

The Nile blue test was described to us by W.A. Yanko, County Sanitation District of Los Angeles County, San Jose Creek Water Quality Laboratory, Whittier, California. His results (not published) have shown that ascaris ova absorbing the Nile blue are not capable of embryonating, i.e., are not viable. As a further test we heat treated ascaris ova at room temperature, 53, 55, and 57°C for periods of 15 and 30 minutes and incubated the ova to check ability to embryonate in the presence and absence of Nile blue.

The results of our Nile blue study showed that no ascaris taking up Nile blue would embryonate and that ova embryonated just as well in the presence or absence of Nile blue, indicating that in itself Nile blue was not toxic to the ova. Apparently uptake of Nile blue indicates that the membrane may be

TABLE 69. COUNTS OF ASCARIS OVA AND PERCENT ACTIVE ASCARIS IN SLUDGE OF EXPERIMENT 4

Week	% Moisture of Sludge	No. of Ascaris/Gram	% Infective
0	91.8	7,056	86.7
13	57.9	2,086	80.0
16	20.4	1,150	79.4
19	7.7	255	3.85
22	4.7	615	0.
28	4.2	443	0.3

functional preventing uptake of the stain, but other defects may prevent embryonation. Therefore, the Nile blue test is a conservative estimate of the number of ova not capable of embryonating and becoming viable.

These results indicate that drying of the sludge to about 10% moisture will inactivate all but a small percent of the ova. Continued drying to about 5% will essentially complete the inactivation of the remaining ova, at least, as determined by the Nile blue test.

11/11/11

11/11/11

LITERATURE CITED

- Adams, M.H. 1959. Bacteriophages, Interscience Publ., Inc., New York. p. 29-30
- Allcroft, R. 1951. Lead poisoning in cattle and sheep. Vet. Rec. 63:583-590.
- Ammerman, C.B., S.M. Miller, K.R. Fickand, S.L. Hansard, II. 1977. Contaminating elements in mineral supplements and potential toxicity: a review. J. Ani. Sci. 44:485-508.
- Anke, M., A. Hennig, H.J. Schneider, H. Ludke, and W. von Gagern. 1970. The interrelationships between cadmium, zinc, copper, and iron in metabolism of hens, ruminants and man. p. 317-320 In: Trace Element Metabolism in Animals. Mills, C.F. (ed.), B. and S. Livingston, Edinburgh and London.
- Anonymous. 1977. Feasibility of using sewage sludge for plant and animal production. Cooperative research project University of Maryland and U.S. Dept. of Agriculture. Washington Suburban Sanitary Commission. Maryland Contract No. 2JAACC-H/2JAAQB-G/2JAANA/28-XXCA.
- Anonymous. 1979a. New Mexico State University (NMSU) agronomic and refeeding studies. In: Progress Report, Beneficial Uses Program, Period Ending June 30, 1978. U.S. Dept of Energy. Contract AT (29-1)-789. National Technical Information Service, Springfield, VA.
- Anonymous. 1979b. Feasibility of using sewage sludge for plant and animal production. Cooperative research project, University of Maryland and U.S. Dept. of Agriculture. Washington Suburban Sanitary Commission. Maryland Contract No. 2JAACC-H/2JAAQB-G/2JAANA/28-XXCA.
- Anthony, W.B. 1974. Nutritional value of cattle. In: Proceedings Symp. on Nutritional Potential for Recycling Waste. Fed. Proc. 33:1933.
- Allaway, W.H. 1968. Agronomic controls over environmental cycling of trace elements. Adv. Agron. 20:235-274.
- Avrahami, M. and R.T. Steele. 1972. Hexachlorobenzene. I. Accumulation and elimination of HCB in sheep after oral dosing. J. Agr. Res. 15:476.
- Baxter, J.C., W.J. Martin, B.R. Sabey, W.E. Hart, D.B. Cohen, and C.F. Calkins. 1977. Comprehensive summary of sludge disposal recycling history. Environmental Protection Technology Series, EPA-600/2-77-054.

Bertrand, J.E., M.C. Lutrick, H.L. Breland and R.L. West. 1978. Dried liquid digested sludge in the ration of feedlot steers. p. 441-450. In: Proc. 1st Annual Conf. of Applied Research and Practice on Municipal and Industrial Waste. Madison, WI.

Bertrand, J.E., M.C. Lutrick, H.L. Breland and R.L. West. 1980. Effects of dried digested sludge and corn grown on soil treated with liquid digested sludge on performance, carcass quality and tissue residues in beef steers. *J. Anim. Sci.* 50:35-40.

Black, C.A., D.D. Evans, J.L. White, L.E. Ensminger, and F.E. Clark (eds.). 1965. In: Methods of Soil Analysis, Chemical and Microbiological Properties. Agronomy No. 9, Part 2, American Society of Agron., Inc.

Bovard, K.P., J.P. Fontonot and B.M. Priode. 1971. Accumulation and dissipation of heptachlor residues in fattening steers. *J. Anim. Sci.* 33:127-132.

Boyle, E.A. and J.M. Edmond. 1975. Determination of trace metals in aqueous solution by APDC chelate co-precipitation. p. 44-55 In: Analytical Methods in Oceanography. T.R.P. Gibb, Jr. (ed.). Advances in Chemistry series 147, American Chemical Society, Washington, D.C.

Burge, W.D. and P.B. Marsh. 1978. Infectious disease hazards of landspreading sewage wastes. *J. of Environ. Qual.* 7:1-9.

Calvert, C.C. 1975. Arsenicals in animal feeds and wastes. p. 70-80 In: Arsenicals Pesticides. E.A. Woolson (ed.). ACS Symposium Series, American Chemical Series, American Chemical Society, Washington, D.C.

Chaney, R.L. 1973. Crop and food chain effects of toxic elements in sludges and effluents. p. 129-141 In: Proceedings of the Joint Conference of Recycling Municipal Sludges and Effluents on Land. Nat. Assoc. St. Univ. & Land-Grant Coll.

Chaney, R.L. 1975. Plant uptake of heavy metals from sewage sludge applied to land. p. 169-178 In: Proceedings of the 1975 Nat. Conf. on Municipal Sludge Management and Disposal. Information Transfer, Inc., Rockville, MD.

Chaney, R.L., S.B. Hornick, and P.W. Simon. 1976. Heavy metal relationships during land utilization of sewage sludge in the Northeast. In: Proceedings of the 8th Annual Waste Management Conference. Cornell University, Rochester, NY.

Chaney, R.L. 1980. Health risks associated with toxic metals in municipal sludge. p. 59-83 In: Health Risks of Land Application. Ann Arbor Science.

Chaney, R.L., S.B. Hornick. 1977. Accumulation and effects of cadmium on crops. p. 125-139 In: Proceedings First International Cadmium Conference. Cadmium Council, NY.

Chaney, R.L., G.S. Stoewsand, A.K. Furr, C.A. Bache, and D.J. Lisk. 1978. Elemental content of tissues of guinea pigs fed swiss chard grown on municipal sewage sludge amended soils. *J. Agr. Food Chem.* 26:994-997.

Code of Federal Regulations, Title 21, Food and Drugs. 1977. Parts 100 to 199. Revised as of April 1, U.S. Government Printing Office, Washington, D.C. 20402.

Council of Agricultural Scientists and Technologists (CAST). 1976. Application of sewage sludge to crop land: Appraisal of potential hazards of the heavy metals to plants and animals. Report No. 64.

Cousins, R.J., A.K. Barber, and J.R. Trout. 1973. Cadmium toxicity in growing swine. *J. Nutr.* 103:964-972.

Crenshaw, G.L. and H.W. Lakin. 1974. A sensitive and rapid method for the determination of trace amounts of selenium in geologic materials. *J. of Res. the U.S. Geol. Surv.* 2:483-487.

Cromartie, E., W.L. Reichel, et al. 1973. Residues of Organochlorine Pesticides and PCB's and Autopsy Data for Bald Eagles, 1971, 1972. The U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, MD 20811.

Cunningham, I.J. 1946. Copper deficiency in cattle and sheep on peat lands. *N.Z. J. Sci. Tech.* 27:381-396.

Dale, W.E., A. Curley, and C. Cueto. 1966. Hexane extractable chlorinated insecticides in human blood. *Life Sci.* 5:47

Davison, K.L. 1970. Dieldrin accumulation in tissues of sheep. *J. Agric. Food Chem.* 18:1156-1160.

Decker, L.B., R.U. Byerrum, C.R. Decker, C.A. Coppert, and R.F. Langham. 1958. Chronic toxicity studies: 1. Cadmium administered in drinking water to rats. *AMA Arch. Ind. Health* 18:228.

de Faubert Maunder, M.J., H. Egan, E.W. Godly, W.E. Hammond, J. Roburn, and J. Thompson. 1964. Clean up of animal fats and dairy products for the analyses of chlorinated pesticide residues. *The Analyst.* 89:168.

Dick, A.T. 1952. The effect of diet and of molybdenum on copper metabolism in sheep. *Australian Vet. J.* 28:30-35.

Dowdy, R.H. 1981. Personal communication. USDA, ARS. University of Minnesota, ST. Paul.

Doyle, J.J., W.H. Pfander, S.E. Grebing, and J.O. Pierce. 1974. Effects of dietary cadmium on growth, cadmium absorption, and cadmium tissue levels in growing lambs. *J. Nutr.* 104:160-166.

- Doyle, J.J. and W.H. Pfander. 1975. Interaction of cadmium with copper, iron, zinc, and manganese in ovine tissues. *J. Nutr.* 105:599-605.
- Doyle, J.J. 1977. Effects of low levels of dietary cadmium in animals - A review. *J. Environ. Qual.* 6:2:111-115.
- Doyle, J.J. 1979. Toxic and essential elements in bone - A review. *J. Animal Sci.* 49:2:482-497.
- Elinder, C.G., M. Piscator, and L. Linnman. 1977. Cadmium and zinc relationships in kidney cortex, liver and pancreas. *Environ. Res.* 13:432-440.
- Elinder, C.G., M. Piscator. 1978. Cadmium and zinc in horses. To be published in *Skall publiceras i Proc. 3rd Int. Symp. Trace element metabolism in Animals and Man.*
- Environmental Protection Agency. 1974a. Methods for Chemical Analysis of Water and Wastes, EPA-625/6-74-003.
- Environmental Protection Agency. 1974b. Analysis of Pesticide Residues in Human and Environmental Samples. Pesticides and Toxic Substances Effects Laboratory, National Environmental Research Center, U.S. EPA Research Triangle Park, NC.
- Environmental Protection Agency. 1977. Tech. Bull. Municipal Sludge Management: Environmental Factors. U.S.E.P.A. Document No. 430/9-77-004. Appendix IX.
- Environmental Protection Agency. 1979. Criteria for classification of solid waste disposal facilities and practices. *Federal Register.* 44:179:53438-53468.
- Federal Register. 1973. June, Vol. 38, No. 125, 29, Part II.
- Feldman, C. 1974. Perchloric acid procedure for wet-ashing organics for the determination of mercury (and other metals). *Anal. Chem.* 46:11:1606-1609.
- Firth, J.A. and B.C. Johnson. 1955. Sewage sludge as a feed ingredient for swine and poultry. *Agr. and Food Chem.* 3:795-796.
- Fitzgerald, P.R. 1980. Observations on the health of some animals exposed to anaerobically digested sludge originating in the Metropolitan Sanitary District of Greater Chicago system. p. 267-284. In: Sludge - Health Risks of Land Application. G. Britton et al. (ed). Ann Arbor Science Publishers Inc., Ann Arbor, MI.
- Flanagan, P.R., J.S. McLellan, J. Hoist, M. Cherian, M.J. Chamberlain and L.S. Valberg. 1978. Increased dietary cadmium absorption in mice and human subjects with iron deficiency. *Gastroenterol.* 74:841-846.

Food and Drug Administration. 1981. Polychlorinated Biphenyl (PCB) Residues; Action Level in Red-Meat Animals. Fed. Reg. 46:147:39224.

Fox, M.R.S. 1976. Cadmium metabolism - A review of aspects pertinent to evaluating dietary cadmium intake by man. p. 401 In: Trace Elements and Human Health and Disease. Vol. 2. A.S. Prasad (ed.) Academic Press. N.Y.

Friberg, L., M. Piscator, G.F. Nordberg, Tord Kjellstrom. 1974. Cadmium in the Environment. 2nd ed. CRC Press, Inc.

Fries, G.F., G.S. Marrow and C.H. Gordon. 1973. Long-term studies of residue retention and excretions by cows fed a polychlorinated biphenyl (AR-1254). J. Agr. Food Chem. 21:117-121.

Fries, G.F. 1977. The kinetics of halogenated hydrocarbons retention and elimination in dairy cattle. p. 159-173. In: Fate of Pesticides in the Large Animal. Academic Press, Inc.

Fries, G.F. and G.S. Marrow. 1977. Distribution of hexachlorobenzene residues in beef steers. J. of Ani. Sci. 45:1160-1165.

Fries, G.F. and G.S. Marrow. 1978. Uptake of chlorobiphenyls by soybean plants. Presented at the 175th Meeting of the American Chemical Society.

Fries, G.F. 1980. An assessment of potential residues in animal products from application of sewage sludge containing polychlorinated biphenyls to agricultural land. Presented at Symposium Sludge-Health Risk of Land Application. Tampa, FL.

Fulkerson, W. and H.E. Goeller (eds.). 1973. Cadmium the Dissipated Element. Oak Ridge Nat. Lab., Oak Ridge, TN. NSF-EP-21.

Furr, A.K., A.W. Lawrence, S.S.S. Tong, M.C. Grandolfo, R.A. Hoptader, C.A. Bache, W.H. Gutenmann, and D.J. Lisk. 1976. Multi-element and chlorinated hydrocarbon analysis of municipal sewage sludges of American cities. Environ. Sci. & Tech. 10:683-687.

Goodrich, R.D. and A.D. Tillman. 1966. Copper, sulfate and molybdenum interrelationships in sheep. J. Nutr. 90:76-80.

Goulden, P.D. and P. Brooksbank. 1974. Automated atomic absorption determination of arsenic, antimony, and selenium in natural waters. Anal. Chem. 46:11:1431-1436.

Hackler, L.R., A.L. Neuman and B.C. Johnson. 1957. Feed from sewage. III. Dried activated sewage sludge as a nitrogen source for sheep. J. Ani. Sci. 16:125-129.

Hambidge, K.M. 1974. Zinc deficiency in children. p. 171-183 In: Trace Element Metabolism in Animal. - 2. (eds.) W.G. Hoekstra, J.W. Suttie, H.E. Ganther, W. Mertz. Univ. Press, Baltimore, MD.

- Harris, C.R. 1970. Persistence and behavior of soil insecticides. p. 58-63. In: Symposium on Pesticides in Soil: Ecology, Degradation, Movement. Michigan State University, East Lansing.
- Harrison, D.L., J.C.M. Mol, and W.B. Healy. 1970. DDT residues in sheep from the ingestion of soil. N.Z.J. Agri. Res. 13:664-672.
- Hatch, W.R., and W.L. Ott. 1968. Determination of sub-microgram quantities of mercury by atomic absorption spectrophotometry. Analytical Chemistry. Vol. 40, No. 14.
- Hays, B.D. 1977. Potential for parasitic disease transmission with land application of sewage plant effluents and sludges. Water Res. 11:583-595.
- Healy, W.B. 1968. Ingestion of soil by dairy cows. N.Z. J. Agri. Res. 11:487-499.
- Healy, W.B. 1970. Ingested soil as a possible source of elements for grazing animals. Proc. N.A. Soc. of Animal Prod. 30:11-19.
- Healy, W.B. 1972. Ingested soil and animal nutrition. Proc. of N. Z. Grassland Assoc. 34:84-90.
- Healy, W.B. 1974. Ingested soil as a source elements to grazing animals. p. 448-450 In: Trace Element Metabolism in Animals - 2. (eds.) E.G. Hoestra, J.W. Suttie, W. Mertz, University Press, Baltimore, MD.
- Hembrey, F.G., L.I. Smart, T.D. Binder and J.M. Dixon. 1975. Hexachlorobenzene decontamination of beef cattle. J. Animal Sci. 41:269.
- Henderson, S.J., DeBoer, J.G., and Stahr, H.H. 1971. Improved method for the determination of chlorinated hydrocarbon pesticide residue in whole blood. Anal. Chem. 43:445-447.
- Hill, C.H., G. Matrone, W.L. Payne, and C.W. Barber. 1963. In Vivo interactions of cadmium with copper, zinc, and iron. J. Nutr. 80:227-235.
- Ingram, B.L. 1970. Determination of fluoride in silicate rocks without separation of aluminum using a specific ion electrode. Anal. Chem. 42:14:1825-1827.
- Jacobs, L.W. 1976. Fate of polybrominated biphenyls (PBB's) in soils. Persistence and plant uptake. J. Agric. Food Chem. 24:1198.
- Jacobs, R.M., M.R.S. Fox, B.E. Fry, and B.F. Harland. 1973. The effect of a two day exposure to dietary cadmium on the concentration of elements in duodenal tissue of Japanese Quail. p. 684-686 In: Proceedings 2nd Inter. Symp. on Trace Element Metabolism in Animals. Madison, WI.
- Jelinek, C.F. and G.L. Braude. 1977. Management of sludge use on land: FDA considerations. p. 35-38 In: Proceedings of the Third National Conference

on Sludge Management, Disposal and Utilization. Inf. Transfer Inc., Rockville, MD.

Johnsen, R.E., and R.I. Starr. 1972. Ultra rapid extraction of insecticides from soil using a new ultrasonic technique. J. Agr. Food Chem. 20:48.

Larkin, E.P., J.T. Tierney, and R. Sullivan. 1976. Persistence of virus on sewage-irrigated vegetables. J. Envir. Eng. Proc., Am. Soc. Civ. Eng. 1:29-35

Lindsay, W.L. 1976. J. Hodgson, and W.A. Norvell. 1966. The physiochemical equilibrium of metal chelates in soils and their influence on the availability of micronutrient cations. Trans. Comm. II & IV. Int. Soc. Soil Sci., Aberdeen. p. 305-316.

Lindsay, W.L. and W.A. Norvell. 1969. Equilibrium relationships of Zn^{2+} , Fe^{3+} , Ca^{2+} , and H^+ with EDTA and DTPA in soils. Soil Sci. Soc. Amer. Proc. 33:1:62-68.

Lindsay, W.L. and W.A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Amer. J. 42:421-428.

Lloyd, M.K. 1970. Fluorine in bones of grazing animals in non-industrial areas. p. 487-490. In: Trace Element Metabolism in Animals. (ed.) C.F. Mills, B&S Livingston, Edinburgh, London.

Loeb, T., and N.D. Zinder. 1961. A bacteriophage containing RNA. Proc. Natl. Acad. Sci. U.S. 47:282-289.

Ludwick, A.E., P.N. Soltanpour, and J.O. Reuss. 1977. Nitrate distribution and variability in irrigated fields of northeastern Colorado. Agron. J. 69:710-713.

McLellan, J.S., P.R. Flanagan, M.J. Chamberlain, and L.S. Valberg. 1978. Measurement of dietary cadmium absorption in humans. J. Toxicol. Environ. Health. 4:131-138.

Mills, C.F. and A.C. Dalgarno. 1972. Copper and zinc status of ewes and lambs receiving increased dietary concentrations of cadmium. Nature 239:171-173.

Mills, P.A., J.H. Onley, and R.A. Gaither. 1963. Rapid method for chlorinated pesticide residues in nonfatty foods. J.A.O.A.C. 46:186-191.

Milner, G.W.C. and G. Phillips. 1967. Coulometry in Analytical Chemistry. Plenum Press, New York.

Moulton, R., P.F. Trowbridge, and L.D. Haigh. 1922. Studies in animal nutrition. II. Changes in proportions of carcass and offal on different planes of nutrition. Agri. Exp. Sta. Res. Bull. #54. University of Missouri.

Nash, R.G. and E.A. Woolson. 1967. Persistence of chlorinated hydrocarbon

insectides in soils. *Science* 157:924-927.

Nash, R.G. 1974. Plant uptake of insecticides, fungicides, and fumigants from soils. p. 257-313. In: *Pesticides in Soil and Water*. W.D. Guenzi (ed.). Soil Science Society of American, Inc., Madison, WI.

Neathery, M.W. and W.J. Miller. 1975. Metabolism and toxicity of cadmium, mercury, and lead in animals: A review. *J. Dairy Sci.* 58:1767-1781.

Neathery, M.W. and W.J. Miller. 1976. The maximum safe dietary levels of nine essential trace elements for cattle. p. 94 In: Proceedings of the 71st Annual Meeting of the American Dairy Assoc.

Norvell, W.A. and W.L. Lindsay. 1972. Reactions of DTPA chelates of iron, zinc, copper, and manganese with soils. *Soil Sci. Soc. Amer. Proc.* 36:778-783.

N.R.C. 1970. *Nutrient Requirements of Beef Cattle*. Fourth rev. ed., NAS:NRC. Print and Publ. Off., NAS, 2101 Constitution Avenue, Washington, D.C. 20418.

Olson, O.E. 1969. Fluorometric analysis of selenium in plants. *J. Assoc. Official Analytical Chemists.* 52:627-634.

Organization for Economic Cooperation and Development (OECD). 1975. *Cadmium and the Environment: Toxicity, Economy, Control*. Paris, France.

Osuna, O., G.T. Edds, J.A. Popp, J. Monague and K.E. Ferslew. 1979. Feeding trials of dried urban sludge and the equivalent cadmium level in swine. p. 201-213 In: Proceedings of Eighth National Conference on Municipal Sludge Management. Information Transfer, Inc. Silver Spring, MD.

Pahren, H.R., J.B. Lucas, J.A. Ryan, and G.K. Dotson. 1977. An appraisal of the relative health risks associated with land application of municipal sludge. *Jour. Water Poll. Control Fed.* 51:2588-2601.

Parizek, J. 1957. The destructive effect of the cadmium ion on testicular tissue and its prevention by zinc. *J. Endocrinology*, 15:56-63.

Parizek, J., Jikalouskova, A. Babicky, J. Benes, and L. Pavlik. 1974. Interaction of selenium with mercury, cadmium, and other toxic metals. pp. 119-131. In: Trace Element Metabolism in Animals - 2. (eds.) W.G. Hoekstra, J.W. Suttie, H.E. Ganther, W. Mertz. University Press, Baltimore.

Patterson, J.B.E. 1971. Metal toxicities arising from industry. *Tech Bull.* Ministry of Agri. Fisheries and Food, Agri. Development and Advisory Services. Cambridge, England. 21:193-207.

Perry, H.M., Jr. 1976. Review of hypertension induced in animals by chronic ingestion of cadmium. p.417-430 In: Trace Elements and Human Health and Disease. Vol. 2 A.S. Prasad (ed.) Academic Press, NY.

Petering, H.G., M.A. Johnson, and K.L. Stemmer. 1971. Studies on zinc metabolism in the rat. Arch. Environ. Health 23:93-101.

Petering, H.G. 1974. The effect of cadmium and lead on copper and zinc metabolism. In: Trace Element Metabolism in Animals - 2. (eds.) W.G. Hoekstra, J.W. Suttie, H.E. Ganther, W. Mertz. University Press, Baltimore.

Piscator, M. and B. Lind. 1972. Calcium, zinc, copper, and lead in renal cortex. Arch. Environ. Health. 24:426-431.

Piscator, M. 1974. Cadmium-Zinc interactions. p. 951-958. In: Proceedings Recent Advances in the Assessment of Health Effects of Environmental Pollution. CEC-EPA-WHO Symposium Paris.

Piscator, M. 1976. The chronic toxicity of cadmium. p. 431-441 In: Trace Elements and Human Health and Disease. Vol. 2. A.S. Prasad (ed.) Academic press. NY.

Polyak, L. Ya. 1962. Titanium in rocks and ores. Shur. NaI. Khin. 17:206.

Powel, G.W., W.J. Miller, J.D. Morton, and C.M. Clifton. 1964. Influence of dietary cadmium level and supplemental zinc on cadmium toxicity in the bovine. J. Nutr. 84:205-214.

Reuss, J.O., P.N. Soltanpour, and A.E. Ludwick. 1977. Sampling distribution of nitrates in irrigated fields. Agron. J. 69:588-592.

Robson, S.G. 1977. Ground-water Near a Sewage-Sludge Recycling Site and a Landfill Near Denver, Colorado. U.S. Geological Survey. Water-Resources Investigations. p. 76-132.

Schroeder, H.A., and W.H. Vinton. 1962. Hypertension induced in rats by small doses of cadmium. Am J. Physiol. 202:515-518.

Schroeder, H.A. and I.H. Tipton. 1968. The human body burden of lead. Arch. Environ. Health. 17:965.

Smith, L.W., G.G. Fries, and B.T. Weinland. 1976. Poultry excreta containing biphenyls as a protein supplement for lactating cows. J. Dairy Sci. 59:465-474.

Smith, G.S., H.W. Kiesling, J.M. Cadle, C. Staples, L.B. Bruce, and H.D. Sivinski. 1976. Recycling sewage solids as feedstuffs for livestock. p. 119-127. In: Proceedings of the 3rd National Conference on Sludge Management Disposal and Utilization. Inf. Transfer Inc., Rockville, MD.

Smith, G.S., H.W. Kiesling, and H.D. Sivinski. 1977. Recycling sewage solids as feedstuffs. Nutrient usage and heavy metals uptake by sheep fed thermoradiated, undigested sewage solids. In: Proceedings of Ninth Annual Waste Management Conference. Cornell University, NY.

Smith, G.S., H.W. Kiesling and E.E. Ray. 1979. Prospective usage of sewage

solids as feed for cattle. p. 190-200. In: Municipal Sludge Management: Impact of Industrial Toxic Materials on POTW Sludge. Proceedings 8th Nat'l. Conference Information Transfer, Inc. Silver Spring, MD.

Smith, G.S., H.E. Kiesling, C.H. Herbel, E.E. Ray, P. Trujillo, R. Orcasberro and J.S. Sivinski. 1980. Sewage solids as supplemental feed for ruminants grazing rangeland forage. Publication S.P. 132. New Mexico State University Agricultural Experiment Station.

Sommers, L.E. 1977. Chemical composition of sewage sludges and analysis of their potential use as fertilizers. J. Environ. Qual. 6:225-232.

Standard Methods for the Examination of water and waste water. 1976. 14th Ed. American Public Health Association. Washington, D.C.

Suttle, N.F. and C.F. Mills. 1966. Studies of the toxicity of copper to pigs. 1. Effects of oral supplements of zinc and iron salts on the development of copper toxicosis. Br. J. Nutr. 20:137-148.

Suzuki, M. 1977. Translocation of polychlorobiphenyls in soil into plants: A study of a method of culture of soybean sprouts. Arch. Env. Contam. and Toxicol. 5:343.

Supplee, W.C. 1963. Antagonistic relationship between dietary cadmium and zinc. Science. 139:121-122.

Thomason, B.M., J.W. Biddle, and W.B. Cherry. 1975. Detection of salmonellae in the environment. Appl. Microbiol. 30:764-767.

U.S. Department of Agriculture, Pesticide Regulation Division. 1968. Summary of registered pesticide chemical uses. Looseleaf N.B.

U.S. Geological Survey. 1963. Analytical Methods Used in Geochemical Exploration by the U.S.G.S. Bull. 1152.

U.S. Geological Survey. 1970. Techniques of Water-Resources Investigations of the United States Geological Survey. Book 5, Chapter A1.

Underwood, E.J. 1977. Trace Elements in Human and Animal Nutrition. Acad. Press. New York, NY.

Van Campen, D.R. and P.V. Scafie. 1967. Zinc interference with copper absorption in rats. J. Nutr. 91:473-476.

Van Campen, D.R. 1970. Copper interference with the intestinal absorption of zinc - 65 by rats. J. Nutr. 97:104-108.

Vanselow, A.P. 1966. Nickel. p. 302-309. In: Diagnostic Criteria for Plants and Soils. (ed.) H.D. Chapman, University of California, Div. of Agri.

Verango, O. and J.G. Hunter. 1953. Nickel and cobalt toxicities in oat plants. *Ann. Botany*. 17:317-328.

Viets, F.G., Jr. and W.L. Lindsay. 1973. Testing soils for Zn, Cu, Mn, and Fe. In: *Soil Testing and Plant Analysis*. (ed.) L.M. Walsh, J.D. Beaton. Soil Science Society of America, Inc., Madison, WI.

Walsh, L.M. and D.R. Keeney. 1975. Behavior and phytotoxicity of inorganic arsenicals in soils. p. 35-52. In: *Arsenical Pesticides*. (ed.) E.A. Woolson. ACS Symposium Series. American Chemical Society. Wash. D.C.

Ward, A.M. and D. Seckler. 1975. Protein and energy conservation of poultry and fractionated animal waste. In: *Energy Agricultural and Waste Management*. (ed.) W.J. Jewel. Ann Arbor Science. Ann Arbor, MI.

Ward, R.L. and C.S. Ashley. 1977. Inactivation of enteric viruses in wastewater sludge through dewatering by evaporation. *Appl. Environ. Microbiol.* 34:564-570.

Webb, M. 1972. Protection by zinc against cadmium toxicity. *Biochem. Pharmacol.* 21:2767-2771.

Wellings, F.M., A.L. Lewis, and C.W. Mountain. 1974. Virus survival following wastewater spray irrigation of sandy soil. p. 253-260. In: *Virus Survival in Water and Wastewater Systems*. (eds.) J.F. Molina, Jr., and B.P. Sagik. Center for Res. in Water Resources. Univ. of Texas, Austin, TX.

Wellings, R.M., A.L. Lewis, and C.W. Mountain. 1976. Demonstration of Solids-associated virus in wastewater and sludge. *J. Appl. Env. Microbiol.* 31:354-358.

Willet, L.B. 1975. Ecretory behavior of polychlorinated biphenyls in lactating cows fed normal and thyroprotein containing rations. *J. Dairy Sci.* 58:765.

Woolson, E.A. 1973. Arsenic toxicity and uptake in six vegetable crops. *Weed Sci.* 21:524-527.

Woolson, E.A. 1975. *Arsenical Pesticides*. ACS Symposium. Amer. Chem. Soc., Wash. D.C.

APPENDIX A

METHODS OF ANALYSIS

ELEMENTAL ANALYSIS OF BEEF CATTLE TISSUE

Samples of the various animal tissues were collected during slaughter. Each sample was collected using stainless steel knives and extreme caution to avoid contamination with gut or foreign material. Whole kidneys were taken for analysis while slices of other tissues were taken transversely through each organ to obtain a composite sample of the whole organ. Bone tissues were tarsal samples from the lower front leg. No attempt was made to remove bone marrow. Tissue samples were rinsed in deionized water and stored in acid washed plastic containers for heavy metals analysis. Identical samples were stored in acid washed glass containers covered with aluminum foil for refractory organics analysis. All samples were stored frozen until analysis.

Cattle tissues from the 3-month Ft. Collins and Metro Denver sludge feeding studies and cattle from the Lowry Bombing Range were analyzed using the following procedure. When the tissue samples were received in the lab they were chilled in liquid nitrogen and then pounded (wrapped in plastic bags) until the sample had shattered into many small pieces. These pieces were then coned and quartered. Representative aliquots were taken for moisture determination, heavy metal analyses, and fluoride analysis when necessary. Percent moisture determinations permitted wet weight results to be converted to a dry weight basis. The tissue samples were then digested in 15 ml nitric and 15 ml perchloric acid with potassium dichromate as a catalyst (Feldman, 1974). An air condenser was used to prevent mercury loss. Copper and zinc were determined directly by flame atomic absorption spectrophotometry (A.A.S.). Arsenic and selenium were analyzed by eliminating nitric acid from an aliquot to reduce the metals to a +3 oxidation state. The metal concentrations were then determined by hydride generation (Goulden and Brooksbank, 1974). Mercury was determined by the cold vapor method for mercury in water (U.S. Geological Survey, 1970). Cadmium, molybdenum, nickel, and lead were determined directly by flame atomic absorption when kidney and liver samples were involved. Concentrations in other tissues were too low for direct determination by flame AAS. Therefore, these samples were preconcentrated after perchloric-nitric acid digestion. Preconcentration involved adding 10 ml of 1% ammonium-pyrollidine dithiocarbamate (APDC), adjusting to pH3 with NH_4OH and isolating the APDC chelate on a 0.22 Millipore filter pad (Boyle et al., 1975). The pad was then stored for later analysis. At the time of analysis the pad with APDC was dissolved in simmering nitric acid and diluted to 10 ml

volume. Metals were then determined directly by flameless A.A.S.

Tissues other than kidney and liver were digested with triple distilled perchloric acid because lead and nickel contaminants in the perchloric acid generally exceeded tissue concentrations. The atomic absorption spectrograph was equipped with a background correction deuterium lamp which was utilized for all metals.

Kidney and liver tissues from the 9-month Ft. Collins sludge feeding trial and the control study examining older cattle of known age were analyzed utilizing a slightly different method than described above. Whole kidneys and 0.5 to 1 kg samples of liver were homogenized in a blender. Aliquots of the homogenized kidney or liver tissue were transferred to tared 100 ml volumetric flasks to give a dry weight of 1 to 2 grams. The aliquot was oven dried at 105°C. 10 ml of concentrated HNO_3 was added and allowed to digest for 2 hours at room temperature. The flasks were placed on a hot plate (163-191°C) and digested for 45 minutes. Samples were cooled and 5 ml of concentrated H_2SO_4 (suitable for Hg determinations) added. The samples were then placed on the hot plate (260-288°C) for 45 minutes. Samples were cooled and 10 ml of concentrated HNO_3 were added, then returned to the hot plate for 60 minutes. Samples were cooled again and 10 ml of concentrated HNO_3 acid added. Samples were then returned to the hot plate until the solution turned clear yellow or orange and for 5 minutes thereafter. The digestate was transferred to a 25 ml volumetric flask and diluted to 25 ml. Samples were then directly determined using flame A.A.S. Lead and Cd analyses of liver and bone biopsies were analyzed using flameless furnace A.A.S. techniques.

Bone samples were analyzed for fluoride content by ashing at 600°C for 3 days. Samples of ash were dissolved in 4 ml of 0.25 N HCl plus 1 ml of concentrated HCl, then diluted with 25 ml deionized H_2O . The pH was adjusted to 6 using NaOH, then brought to a final volume of 50 ml. A 26 ml aliquot was taken, to which 25 ml of buffering solution (pH 6.0 - 6.5) was added. Fluoride potential was then determined using a fluoride sensitive electrode (Ingram, 1970; EPA, 1974a).

ELEMENTAL ANALYSIS OF SOIL AND PLANT TISSUES

Plant samples were washed in a dilute solution of lauryl sulfate, dried, ground to 20 mesh in a stainless steel Wiley mill, coned and quartered, and then stored in plastic bags until analysis. Soil samples were air dried, crushed, sieved through a 32 mesh stainless steel sieve, coned and quartered and stored in plastic bags until analysis.

Extraction of total metals (Zn, Cu, Ni, Cd, Pb, Sn, and Mo) in plant and soil samples was accomplished using the same technique. Samples were oven-dried overnight at 105°C, 1 to 5 grams weighed into 250 ml beakers, and ashed at 480°C for 14 hours. Ashed samples were then refluxed with 10 ml of concentrated HNO_3 and taken to near dryness, followed by addition of 10 ml 3N HCl. Ashed samples were refluxed again and then filtered through #43 acid

washed Whatman filter paper. The filtrate was then volumetrically diluted to 50 ml with deionized water, after which the metals were determined directly with flame A.A.S.

Total arsenic in soils and plant tissues was determined by digestion under reflux in nitric perchloric acids, followed by liberation of arsine gas which was measured by atomic absorption spectrophotometry (U.S. Geological Survey, 1970).

Total selenium in soil and plant tissues was determined by sample digestion in nitric and perchloric acids and selenium content measured fluorometrically (Olson, 1969; Crenshaw and Lakin, 1974).

Total mercury in soil and plant tissues was determined by decomposition of the sample under reflux with perchloric and nitric acids. The mercury was then reduced and measured by the cold vapor technique utilizing atomic absorption spectrophotometry (Hatch and Ott, 1968).

Total titanium was analyzed by fusion of the sample with potassium pyrophosphate, then determined after dissolution with flame atomic absorption spectrophotometry (Polyak, 1962; U.S.G.S., 1963).

Plant "available" heavy metals (Zn, Cu, Ni, Cd, and Pb) were determined by the diethylenetriaminepentaacetic acid (DTPA) procedure of Lindsay and Norvell (1978).

Available soil phosphorus was determined as sodium bicarbonate extractable phosphorus (Black et al., 1965, p. 1035). After extraction the phosphorus was digested with persulfate to produce orthophosphate. Orthophosphate was then determined colorimetrically with a Technicon Autoanalyzer using the molybdenum blue method (EPA, 1974a).

Soil pH was determined with a hydrogen electrode in a 1:1 solution of soil and water. Soil conductivity was determined with a wheatstone bridge on the same 1:1 soil solution after filtration.

Cation exchange capacity was determined by saturating the soil with sodium acetate and replacing sodium with ammonium acetate (Black et al., 1965, p.899). Methanol was used to wash excess sodium from the soil instead of isopropyl alcohol. The exchanged sodium was subsequently determined with a flame photometer.

Soil nitrate and ammonium were extracted with 2N KCl (Black et al., 1965, p. 1185) and determined using a Technicon Autoanalyzer. Nitrate was reduced to nitrite and complexed with N-(1-naphthyl)-ethylenediaminedihydrochloride, which was then determined colorimetrically (EPA, 1974a). Ammonium was determined by reacting with phenol and sodium hypochlorite which produces a blue color; final concentration was then determined colorimetrically (EPA, 1974a).

Total Kjeldahl nitrogen (TKN) content of soils was determined by digestion in sulfuric acid, and distillation of NH_3 into dilute boric acid

(Black et al., 1965, p. 1162). Ammonium in the boric acid solution was then determined with the auto analyzer as reported above.

Percent organic carbon in soils was determined using the Walkley Black method (Black et al., 1965, p. 1372).

PATHOGEN ANALYSIS

Assay for ascaris ova

The following procedure was adapted from an unpublished procedure used in Dr. R.P. Fitzgerald's laboratory, Department of Veterinary Medicine, University of Illinois, Urbana, IL.

The day before analysis of the sludge three 50 ml polycarbonate tubes were carefully filled by layering with 5 ml volumes of 10, 30, 33, 40, and 80% Shaetler's solution (SS). Full strength (100%) SS consisted of 500g sucrose, 320 mg distilled water, and 6.5 g phenol. The solutions were layered by first pipetting the lowest density solution (10%) into the tube and then pipetting the consecutively higher density solutions through the lower density solution into the bottom of the tube. The solution gradients can then be stored for up to three weeks under refrigeration. The layers can be made more distinguishable by the addition of food coloring agents to the different SS solutions.

For analysis an amount of wet sludge to approximate about 0.3 g of solids (dry basis) was added to a blender with 10 ml of 7X (full strength detergent, Limbo Scientific Inc.). Enough distilled water was then added to fill the blender 3/4 full. After blending about 2 min. the slurry was passed through a 40 mesh Tyler screen into a 2-liter beaker. The material left behind on the screen was washed several times. The final volume of the slurry passed through the screen was from one to two liters.

After a 2 to 3 hour settling period the supernatant was carefully discarded, the settled solids weighed into a tared beaker and separated into three subsamples by weight. Each subsample was placed into a 50 ml plastic centrifuge tube which was filled with 10% 7X, shaken and centrifuged for less than 1 minute at about 3,500 g (where g equals gravitational constant). The supernatant was decanted by suction and the solids remaining were again shaken with 4% 7X and again centrifuged. This procedure was repeated until the supernatant was clear. After sucking out the last supernatant, the residual sludge was mixed with 5-10 ml of 10% 7X and transferred by dricote treated pipette to the top of the prepared SS gradient tubes to form a layer on the gradient. Using a swinging bucket head the gradients were centrifuged for 10 minutes at 1,000 g. After centrifugation the 30, 33, and 40% layers which should contain the ova were transferred to a clear plastic tube and enough 10% 7X added to fill the tube 3/4 full. After shaking to mix the gradients, 0.5 ml of 30% SS was pipetted into the bottom of the tube to form a layer. After centrifuging again for 10 minutes at 1,000 g the supernatant

was removed by suction, leaving the ova in the 30% SS layer. The density of the solution was increased for transfer by adding 0.5 ml of 100% SS along with 5 drops of 7X. After aspiration for mixing, the sample was transferred to a Sedgewick Rafter cell of 1.0 cc volume. The volume of any remaining sample is estimated with a 0.1 ml graduated pipette. The ova on a sludge basis is calculated as follows:

$$\text{ova/g} = \frac{\text{ova observed}}{\text{grids counted}} \times 755.86 \text{ grid ml}^{-1} \times \text{ml of final sample volume} \\ \times 3 \text{ wt of sludge}$$

Staining ascaris ova with Nile Blue

Nile Blue sulfate was dissolved in 95% ethanol to form a 1% solution. A volume of this solution was added to the ascaris ova suspension to form a 0.1% solution of Nile Blue. After 15 minutes contact time the ova were examined under a microscope. Ova with dark blue centers were counted as dead. With each set of unknowns, two control tubes were used to standardize the procedure. Both contained 0.5 ml of an ascaris ova standard, 0.5 ml 100% Shaeter's solution, and 5 drops concentrated 7X detergent. One tube was placed in boiling water for 5 minutes and 0.1 ml Nile Blue solution was added to both. The boiled tube contained inactive ascaris ova that stained deep blue. The unboiled tube contained mostly active ascaris ova. Examining slides prepared from these tubes helped eliminate doubt about active vs. inactive ova in the slides prepared from experimental samples.

Growth of f2 and T7

F2 bacterial virus was obtained from the American Type Culture Collection (ATCC #15766-B) as was its host *E. coli* K-13 (ATCC#15776). *E. coli* K-13 was grown according to the method described by Loeb and Zinder (1961) in tryptone yeast extra media (TYE) which contains 10 g tryptone, 8 g NaCl, 1 g yeast extract, 1 g glucose and 0.22 g CaCl_2 per liter. Ten liter batches of f2 were prepared in a New Brunswick M19 Fermenter at 37°C under aerobic conditions. The media was inoculated with an overnight culture of K13 and bacterial growth was monitored by circulating 50 ml/min through a micro flow-through cell in a Beckman DB-G Spectrophotometer at 525 nm. At an absorbance of 0.4 (5×10^8 cells/ml) the appropriate amount of f2 was added to give a multiplicity of infection (MOI) of 0.1. After 2-3 hours when bacterial lysis had stopped, 20 ml of chloroform was added and mixed for 10 minutes. The bacterial debris was removed by centrifugation in a Beckman RC 2B centrifuge at 4°C using a Szent-Gyorgyi and Blum continuous apparatus. The preparation, which averaged 2.0×10^{12} pfu/ml, was stored at 4°C until used in field studies.

Preparations of T7 (ATCC #11303-B7) were done in the same manner as

those described for f2 except that *E. coli* J (ATCC #11303) was used as a host at a MOI of 0.001. The titer of the T7 preparations averaged 8×10^{10} pfu/ml.

Enumeration of f2 and T7 --

Samples were assayed for f2 and T7 by the agar overlay method described by Adams (1959) in 100 mm Petri dishes containing a prepoured layer of hard agar (TYE medium plus 1.5% Bacto agar). The appropriated bacteria and 0.1 ml of the virus dilutions were mixed with 2.7 ml of TYE soft agar (0.7%) and then distributed over the hard agar layer. After the agar had solidified at room temperature, the plates were incubated at 37°C for 6 to 24 hours. All dilutions were plated in duplicate.

Elution of f2 and T7 from sludge --

The viruses were eluted from sludge essentially as described by Wellings et al. (1976). A 30 g sample was weighed into a 250 ml centrifuge bottle and 120 ml of 0.1 M phosphate buffer added. Beef embryo extract was added to make a 3.0% solution and the pH adjusted to 9.0 with 1 N NaOH. The samples were placed in an ice bath and then sonicated at 100W for 15 minutes using a Heat Systems Sonifier W-220F. Debris was removed by a 10 minute spin at 10,000 g and the supernatant fluid adjusted to pH 7.2 and assayed for virus.

Assay for coliforms and salmonellae --

A 50 g sample of sludge was shaken vigorously in a reciprocating shaker for 5 minutes with 450 ml of peptone water (0.1% w/v). The resulting suspension was then used as the initial dilution for the MPN determination of total and fecal coliforms (Standard Methods for the Examination of Water and Wastewater, 1976).

For salmonellae both qualitative and quantitative procedures were used. If the qualitative procedure gave positive results a quantitative procedure was also employed.

For the qualitative procedure the method described by Thomason et al. (1975) was followed. Briefly, 30 g of sludge were added to 100 ml of a buffered peptone solution. After incubation for 18-24 hours at 35-37°C, a 10 ml aliquot was transferred to 100 ml of tetrathionate broth for incubation at 43°C for 18-24 hours. Next the incubated tetrathionate broth was streaked on brilliant green (BG) and bismuth sulfide (BS) agar. Suspect (pink) colonies grew after incubation at 35-37°C for 18-24 hours. Positive cultures that did not agglutinate with saline (they were discarded if they did) were tested serologically with polyvalent O and polyvalent H antisera. Cultures agglutinating with both were considered positive.

For sludge samples positive by the above test, the sludge suspension used for coliform analysis was diluted serially (usually 10^{-1} through 10^{-3} or 10^{-4}) and used to inoculate MPN tubes of buffered peptone water. After incubating 18-24 hours at 35-37°C, 1 ml from each tube was transferred to tetrathionate broth tubes and incubated and tested as in the qualitative procedure for identification of positive tubes. The MPN was determined from the MPN tables in Standard Methods for the Examination of Water and Wastewater, 1976.

Chloride determination

The water samples taken from the ceramic cup lysimeters below the sludge bed were analyzed directly for chlorides by use of the Buchler-Cotlove Chloridometer, Buchler Instruments, Inc. (Millner and Phillips, 1967).

REFRACTORY ORGANIC ANALYSIS

Sampling Method

The Colorado Epidemiologic Pesticides Studies Center (the Center) provided all of the glass containers for collection of samples pertinent to the sludge. All of the glass containers were precleaned and tested before sample collection. All lids and caps were lined with Teflon or aluminum foil.

All samples that were not extracted within 24 hours were deep frozen at -12°C.

The specific refractory organics analyzed included the following compounds:

PCB's	Heptachlor	Mirex
p,p'-DDT	Endrin	Hexachlorobenzene
o,p'-DDT	Thiodan	trans-Nonachlor
p,p'-DDE	Lindane	Aldrin
o,p'-DDE	Dieldrin	Chlordane
p,p'-DDD	Heptachlor Epoxide	Dacthal
alpha-BHC	Oxychlordane	Methoxychlor
beta-BHC		

Analysis of Refractory Organics

The majority of the analytical methodology used in this study was found in a manual primarily intended for use by: EPA laboratories conducting analyses of pesticides in various sectors of the environment; by laboratories

under contract with EPA to conduct community studies; and by laboratories involved in the monitoring of concentrations of pesticides in human populations. The manual was prepared by the Pesticides and Toxic Substances Effects Laboratory, National Environmental Research Center (1974).

Analytical Methods for Liver, Kidney, Fat, and Muscle Tissue

This procedure combines some of the extraction features of the de Faubert Maunder et al. (1964) method and the Florisil partitioning and cleanup basics of the Mills et al. (1963) procedure. The modified procedure has been collaboratively studied over a period of years and has been found to yield interlaboratory relative standard deviation values of 15 percent or better for the chlorinated pesticidal compounds most commonly found in the fat of human and animals.

A 5 gm sample was dry-macerated with sand and anhydrous Na_2SO_4 . Organic residues were then isolated by repetitive extractions with hexane. Pesticides residues were extracted from the sample with acetonitrile and then partitioned back into hexane by aqueous dilution of the acetonitrile extract. The hexane extract was concentrated to 5 ml by Roto-Vap flash evaporation and transferred to a Florisil column for successive elutions with 6%, 15%, and 50% ethyl ether/hexane. The respective eluates both were concentrated to suitable volumes and the final 15% and 50% extracts were examined by electron capture gas-liquid chromatography (EEGC). The 6% extracts were further examined by the silicic acid column chromatographic separation technique for the separation of polychlorinated biphenyls (PCB's) from organochlorine pesticides. Cromartie et al. (1974) have modified the original method to eliminate celite and air pressure in order to accelerate the elution mixture flow rate.

The silicic acid was cleaned and standardized before use as follows: The silicic acid (Mallinckrodt Silic-AR CC-4) was prewashed three times with 50 ml portions of Fraction III elution mixture (ACN/Hexane/MC 1:19:80). It was then heated in an oven for 24 hours at 130° in a beaker covered with aluminum foil containing a few pinholes. After the 24 hour period, the beaker was removed from the oven and cooled to room temperature. A portion of the silicic acid (40 gms) was transferred into a glass stoppered flask. To this was added 1.2l of distilled water to give 3% deactivated silicic acid. The flask was stoppered and wrapped with parafilm, and placed on a shaker for one hour and then allowed to equilibrate for 30 minutes before use.

Twenty gm of the 3% deactivated silicic acid were placed in a beaker, and 70 ml of distilled hexane were added immediately to the beaker to form a slurry. The slurry was added to a chromatographic column to which one-half inch of anhydrous Na_2SO_4 was added to the top of the silicic acid surface, preventing the surface from being disturbed. After using 50 ml of petroleum ether to rinse the column, the 6% extract was transferred to the column with a disposable pipet. Three elution fractions were collected.

Fraction I consisted of 100 ml of petroleum ether that contained the hexachlorobenzene and Mirex.

Fraction II consisted of 300 ml of petroleum ether that contained PCB's, heptachlor, and DDE.

Fraction III consisted of 200 ml of 1:19:80 acetonitrile:hexane:methylene chloride that contained the remaining pesticides.

The elution rates for Fraction I and II were established at ca 5 ml per minute. Fraction III was passed through the column with the stopcock wide open. The eluates of Fractions I and II were concentrated to a suitable volume for analysis of the pesticides and PCB's. Due to the presence of acetonitrile and methylene chloride in Fraction III, it was necessary to reduce the volume to 0.1 ml to remove the polar solvents and then to increase the volume with hexane to a suitable volume for GC analysis.

Analytical Methods for Water

The methodology for the analysis of water incorporated the basic principles and the majority of the specific details of the method appearing in the Federal Register (1973).

Two liters of each sample were transferred to a four liter separatory funnel (No. 1), and 120 ml of 15% methylene chloride/hexane was added. The funnel was stoppered and shaken vigorously for 2 minutes. The layers were allowed to separate, and the aqueous layer was drawn off into a second 2 liter separatory funnel (No. 2).

Another 120 ml of 15% methylene chloride/hexane was added to the aqueous phase in separatory funnel No. 2 after which it was stoppered and shaken vigorously for 2 minutes.

A two-inch column of anhydrous, granular Na_2SO_4 was prepared in a 150 x 24 mm filter tube with a coarse glass fritted disc at the bottom. The column was positioned over a 500 ml flask. The methylene chloride/hexane extract in separatory funnel No. 1 was filtered through the Na_2SO_4 column into the flask.

The aqueous layer in separatory funnel No. 2 was drawn off into the empty separatory funnel No. 1. A volume of 120 ml of hexane was added to the aqueous solution in separatory funnel No. 1, after which it was stoppered and shaken again for 2 minutes. The resultant aqueous layer was drawn off and discarded.

The solvent extracts in both separatory funnels were filtered through the Na_2SO_4 column into the flask, and the filter tube was rinsed with three 10 ml portions of hexane.

The combined extracts were concentrated to 5 ml by Roto-Vap flash evaporation and transferred to a Florisil column for successive elution with 6%, 15%, and 50% ethyl ether/hexane. The respective eluates were concentrated to suitable volumes and the final 15% and 50% extracts were examined by E.C.G.C. The final 6% extracts were further separated by the silicic acid column chromatographic separation technique described earlier.

Analytical Methods for Blood

Since the whole blood samples collected for analysis were frozen immediately after collection, it was not possible to obtain serum by centrifugation. Therefore, the Dale et al. (1966) method found in the manual could not be followed. An alternate method for extractions was followed. This method was essentially that described by Henderson et al. (1971).

A 2 ml aliquot of whole blood was pipetted into a 30 ml glass stoppered centrifuge tube. A 1.5 ml aliquot of 60% sulfuric acid was added to the blood, and the solution was mixed using a Vortex mixer. A second 1.5 ml aliquot of 60% H_2SO_4 was added with mixing, and finally a 2 ml aliquot of 60% H_2SO_4 was added. The solution was again mixed. After cooling for several minutes, 3 ml of acetone/hexane (1:9) were added to the sample. The resulting solution was mixed for 1 minute using a vortex mixer. After centrifuging at 200 rpm to separate the layers, the upper solvent phase was removed using a disposable pipet and placed in a 10 ml concentrator tube. The extraction was repeated two more times with 3 ml of the acetone/hexane solvent, combining the three extracts in the concentrator tube. The combined extracts were evaporated with a gentle stream of nitrogen to less than 1.0 ml, and then brought to exactly 1.0 ml with hexane. Silicic-AR CC-4 (20 mg) was then added to the extract. The tube was inverted several times to mix before analysis by gas chromatography.

Analytical Methods for Brain Tissue

The size of many tissue samples was so minimal that the method described for liver, kidney, muscle, and fat was unsuitable. This procedure, requiring only 0.5 gm, was more suitable for the brain samples collected.

A 0.5 gm sample of brain was extracted in a size 22 dual tissue grinder with 2.5 ml of acetonitrile. The supernatant was poured into a 50 ml round bottom test tube. The extraction was repeated twice more, combining all supernatants in the test tube. Twenty-five ml of 22% aqueous sodium sulfate were added to the contents of the test tube. The contents then were mixed with the aid of a Vortex mixer. The aqueous acetonitrile mixture was extracted with one 5 ml and two 2 ml portions of hexane. The extracts were

combined in a 10 ml evaporative concentrator tube. The extract then was concentrated to a volume of about 0.500 ml with nitrogen. Acetic anhydride (0.3 ml) and pyridine (0.3 ml) were added to the concentrator tube and the tube was placed in a 60-65°C water bath for 30 minutes. After this time, 9 ml of 2% Na₂SO₄ were added and the sample was extracted with 2 to 3 ml portions of hexane three times. The combined extracts were concentrated to approximately 0.3 ml and transferred to the top of a micro Florisil column.

The micro Florisil column was prepared by placing a small loose plug of glass wool in the tip of a size "B" Chromaflex column. The column was packed with a 1.6 gm of 60 to 100% mesh Florisil which had been activated by the manufacturer at 1200°F. The column was washed with 50 ml of hexane followed by 50 ml of methanol. The column was placed in a 130°C oven and conditioned at that temperature overnight before using. The Florisil column was removed from the oven and allowed to cool to room temperature. Finally, the column was prewashed with 10 ml of hexane and the eluate discarded.

Collection of the eluate was started immediately in a 25 ml capacity concentrator tube. Hexane (0.25 ml) was added to the concentrator tube and transferred to the top of the column. This step was repeated a second time. The elution and collection proceeded using 12 ml of hexane followed by 12 ml of 1% methanol in hexane. This 24 ml represented fraction one. A second fraction was collected by eluting with a second 12 ml portion of 3% methanol in hexane. The two fractions were then analyzed by E.C.G.C.

Analytical Methods for Sludge, Feed, Silage, and Soil Samples

The initial analytical method used for extraction of refractory organics from these substances was the continuous Soxhlet extraction technique with acetone/hexane. It was evident from the results of these first experiments that interfering lipid-soluble materials were not effectively removed by successive cleanup on aluminum oxide and Florisil columns. Therefore, a method using a high specific intensity ultrasonic generator was used to extract these substrates. The Polytron (Model PT020ST), an ultrasonic version of the Ultra-Thurraz, was immersed in the sample. This generator was equipped with a saw tooth cutting head which aided in reducing soil particles and aggregates to a very fine "powder". This model has pulse-frequency maximum of 9400 cps (9.4 KHz) and a maximum generator resonator speed of 19500 rpm. Motor speed, and thereby effective frequency, amplitude, and specific intensity, is controlled by a continuously variable rheostat. The Polytron has been used by other investigators to extract soils for insecticides and PCB residues (Johnsen and Starr, 1972).

Two grams of sludge, feed or soil and 5 grams of silage were placed into a large French square bottle. A volume of 100 ml of acetone was added to the bottle and the Polytron's head was immersed into the solution for extraction. The sample was extracted for 30 seconds at maximum power. The acetone extract was filtered through Whatman #1 filter paper into a 2 liter separatory funnel. The extraction with 100 ml of acetone was repeated again, combining the extracts in the same separatory funnel. A third extraction was

accomplished with 100 ml of hexane, again combining this extract with the two acetone extracts in the 2 liter separatory funnel.

A volume of 500 ml of 2% Na_2SO_4 was added and the mixture shaken for 2 minutes. The aqueous acetone phase was drawn off into another separatory funnel and extracted with another 100 ml of hexane. This extraction was repeated a total of three times. The three hexane extracts were combined and extracted one time with 100 ml of 2% Na_2SO_4 .

The total hexane extract was transferred to a rotary evaporative flask and the solvent mixture was removed under water aspiration suction at 37°C until the volume was reduced to approximately 2 ml. The sample was quantitatively transferred to a Florisil column which was prepared in the same manner described previously.

The 6%, 15%, and 50% eluates were collected and the 15% and 50% fractions were analyzed by E.C.G.C.

Detection Limits

The detection limits were determined on a Micro-Tek (MT-220) equipped with paralleled plate, tritium or nickel-63 electron capture detectors, operating in the DC mode. All precoated GC column packings were supplied by the Quality Assurance Section of the EPA. These limits were determined for each substrate and were defined as the concentration of a particular compound in solution when it gave a GC peak equal to one-half the peak height of the routine GC standard used by the Center. The major standards were routinely chromatographed at two different concentrations. These standards and concentrations are shown in table A2. In determining detection limits of compounds that have standards of two different concentrations, the limit was based on the lower concentrated standards. The detection limit was then calculated according to the formula:

$$\text{D.L.} = \frac{(X)(Y)}{Z}$$

where D.L. = detection limit.

X = 1/2 the concentration of the GC Standards. (Note: If two concentrations of A and B are given for a particular compound, X = 1/2 concentration of the lowest concentration of the lowest concentrated standard.)

Y = 1 of standard injected

Z = the amount of sample injected on the GC

This formula was used to calculate the detection limits for the compounds of interest. The results are shown in Tables A3 through A12.

TABLE A1. PERCENT RECOVERY OF ORGANIC COMPOUNDS FROM DIFFERENT SAMPLE TYPES

	Sludge	Feed	Silage	Water	Kidney	Liver	Fat	Muscle	Blood	Brain
alpha-BHC	66	63	90	88	91	96	90	77	94	75
beta-BHC	85	82	82	-	79	85	88	80	88	81
Aldrin	90	96	82	82	-	107	82	85	73	76
Oxychlordan	86	79	90	72	87	88	82	85	90	81
t-Nonachlor	81	83	78	85	86	85	84	86	93	109
Dieldrin	81	91	91	103	-	78	93	94	91	81
o,p'-DDT	89	75	92	99	88	87	88	86	91	79
p,p'-DDT	91	85	80	88	89	88	90	85	92	95
HCB	75	85	76	79	60	75	59	-	-	92
Lindane	69	77	82	105	89	97	79	-	96	92
Heptachlor	96	60	66	54	55	86	73	-	98	104
Hep. Epoxide	71	86	82	85	93	99	84	-	87	103
p,p'-DDE	82	87	86	90	93	104	88	-	95	99
p,p'-DDD	82	89	89	85	97	107	84	-	-	-
Dacthal	96	73	83	89	-	82	90	-	-	-
Thiodan	72	83	82	101	-	76	90	-	-	-
Methoxychlor	92	88	83	74	98	42	109	-	-	-
Chlordane	98	79	89	81	90	92	85	-	73	-
Endrin	92	45	83	101	-	124	98	-	-	-
o,p'-DDE	-	-	89	82	90	96	91	-	-	-
o,p'-DDD	86	94	101	-	89	-	89	-	-	-
Mirex	70	82	76	88	79	93	72	-	-	-
AR-1254	120	89	106	118	-	100	74	-	90	132

TABLE A2. GAS CHROMATOGRAPH STANDARDS

Electron Capture 1	pg/ l		Electron Capture ²	pg/ l	
	A ¹ .	B ² .		A	B
alpha - BHC	4	1	HCB	4	1
beta - BHC	20	5	Lindane	8	2
Aldrin	8	2	Heptachlor	8	2
Oxychlorane	8	2	Aldrin	8	2
t-Nonachlor	8	2	Hept. Expoxide	8	2
Dieldrin	16	4	p,p' - DDE	20	5
o,p' - DDT	40	10	p,p' - DDD	40	10
p,p' - DDT	40	10			
Electron Capture 3		pg/ l	Electron Capture 4		pg/ l
Aldrin		8	Chlordane		50
Dacthal		10	Endrin		10
Thiodan		20			
Methoxychlor		50			
Electron Capture 5		pg/ l	Aroclor		pg/ l
Aldrin		8	1254		250
o,p' - DDE		10	1260		250
o,p' - DDD		20			
Mirex		50			

1. High standard.

2. Low standard.

TABLE A3. DETECTION LIMITS IN PPB (WET WT.) FOR SLUDGE MIXED WITH CATTLE FEED

	Theoretical	Practical
alpha-BHC	2.5	4.0
beta-BHC	12.5	19.0
Heptachlor	5.0	7.5
p,p'-DDT	25.0	40.0
o,p'-DDT	25.0	40.0
p,p'-DDE	12.5	19.0
o,p'-DDE	25.0	40.0
p,p'-DDD	25.0	40.0
Endrin	25.0	40.0
Thiodan	50.0	75.0
Lindane	5.0	7.5
Dieldrin	10.0	15.0
Heptachlor Epoxide	5.0	7.5
Oxychlordane	5.0	7.5
Mirex	125.0	190.0
Hexachlorobenzene	2.5	4.0
t-Nonachlor	5.0	7.5
Aldrin	5.0	7.5
Chlordane	125.0	190.0
Dacthal	25.0	40.0
Methoxychlor	125.0	190.0
Aroclor 1254	625.0	940.0

Based on 2.0 grams extracted with a final volume of 10 ml and 5.0 μ l injected on the GC.

INCHES
FIRST
LINE OF
TEXT
MARG

Typing Guide Sheet

CENTER
OF PAGE

TOP OF
IMAGE
AREA

TABLE A4. DETECTION LIMITS IN PPB FOR DRINKING WATER OF CATTLE

	Theoretical	Practical
alpha-BHC	0.001	0.03
beta-BHC	0.007	0.09
Heptachlor	0.002	0.04
p,p'-DDT	0.01	0.24
o,p'-DDT	0.01	0.24
p,p'-DDE	0.007	0.09
o,p'-DDE	0.01	0.24
p,p'-DDD	0.01	0.24
Endrin	0.01	0.22
Thiodan	0.03	0.11
Lindane	0.002	0.04
Dieldrin	0.005	0.14
Heptachlor Epoxide	0.002	0.06
Oxychlordane	0.002	0.06
Mirex	0.07	0.47
Hexachlorobenzene	0.001	0.03
t-Nonachlor	0.002	0.06
Aldrin	0.002	0.04
Chlordane	0.07	0.47
Dacthal	0.01	0.24
Methoxychlor	0.07	0.47
Aroclor 1254	0.34	1.60

Based on extracting 3700 grams of samples, with a final volume of 10 ml and 5.0 μ l injected on the GC.

TABLE A5. DETECTION LIMITS IN PPB FOR WASTEWATER EFFLUENT SAMPLES

Compound	Theoretical		Practical
	A	B	
p,p'-DDT	0.09	0.02	0.10
o,p'-DDT	0.09	0.02	0.10
p,p'-DDD	0.09	0.02	0.10
Aldrin	0.02	0.01	0.10
Oxychlordane	0.02	0.01	0.10
t-Nonachlor	0.02	0.10	0.10
Lindane	0.02	0.01	0.10
Heptachlor	0.02	0.01	0.10
Heptachlor Epoxide	0.02	0.01	0.10
Dieldrin	0.04	0.01	0.10
alpha-BHC	0.01	0.002	0.05
HCB	0.01	0.002	0.05
beta-BHC	0.05	0.01	0.10
p,p'-DDE	0.05	0.01	0.10
PCB (AR-1254)	2.3	0.6	1.00
Chlordane	0.46	0.13	0.65

A - Samples with volumes below 270 ml. B - Samples with volumes of 990 ml.
Based on 5.0 μ l "plugged" on G.C., 5.0 ml final volume extract.

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

TABLE A6. DETECTION LIMITS IN PPB (WET WEIGHT) FOR SILAGE

	Theoretical	Practical
alpha-BHC	0.1	1.0
beta-BHC	0.5	1.0
Heptachlor	0.2	1.0
p,p'-DDT	1.0	2.0
o,p'-DDT	1.0	2.0
p,p'-DDE	0.5	1.0
o,p'-DDE	1.0	2.0
p,p'-DDD	1.0	2.0
Endrin	1.0	2.0
Thiodan	2.0	3.0
Lindane	0.2	1.0
Dieldrin	0.4	1.0
Heptachlor Epoxide	0.2	1.0
Oxychlordan	0.2	1.0
Mirex	5.0	8.0
Hexachlorobenzene	0.1	1.0
t-Nonachlor	0.2	1.0
Aldrin	0.2	1.0
Chlordane	5.0	8.0
Dacthal	1.0	2.0
Methoxychlor	5.0	8.0
Aroclor 1254	25.0	40.0

Based on extracting 5.0 grams, with a final volume of 1.0 ml, and 5.0 μ l injected on the GC.

TABLE A7. DETECTION IN PPB (WET WT.) FOR FAT, FEED, LIVER AND KIDNEY

	Theoretical	Practical
alpha-BHC	1.2	10
beta-BHC	6.25	10
Heptachlor	2.5	10
p,p'-DDT	12.5	20
o,p'-DDT	12.5	20
p,p'-DDE	6.2	10
o,p'-DDE	12.5	20
p,p'-DDD	12.5	20
Endrin	12.5	20
Thiodan	25.0	35
Lindane	2.5	10
Dieldrin	5.0	10
Heptachlor Epoxide	2.5	10
Oxychlordane	2.5	10
Mirex	62.5	70
Hexachlorobenzene	1.2	10
t-Nonachlor	2.5	10
Aldrin	2.5	10
Chlordan	12.5	70
Dacthal	12.5	20
Methoxychlor	62.5	70
Aroclor 1254	312	500

Based on extracting 4.0 grams of liver or kidney, final volume of 10.0 ml and 5.0 μ l injected on the GC, or 2.0 grams of fat, and feed extracted, final volume of 5.0 ml and 5.0 μ l injected on the GC.

BEGIN
 LINE OF
 TEXT
 HERE

TABLE GUIDE SHEET

CENTER
 OF PAGE

TOP OF
 IMAGE
 AREA

TABLE A8. DETECTION LIMITS IN PPB (WET WEIGHT) FOR BRAIN

DETAIL
 HEAD
 BODY
 SECTION
 INFO

	Theoretical	Practical
alpha-BHC	0.5	1.0
beta-BHC	1.5	2.0
Heptachlor	1.0	2.0
p,p'-DDT	5.0	8.0
o,p'-DDT	5.0	7.0
p,p'-DDE	2.5	5.0
o,p'-DDE	5.0	8.0
p,p'-DDD	3.0	5.0
Endrin	-	-
Thiodan	-	-
Lindane	0.6	1.0
Dieldrin	1.2	2.0
Heptachlor Epoxide	0.6	1.0
Oxychlordane	0.6	1.0
Mirex	-	-
Hexachlorobenzene	0.5	1.0
t-Nonachlor	1.0	2.0
Aldrin	1.0	2.0
Chlordane	-	-
Dacthal	-	-
Methoxychlor	-	-
Aroclor 1254	125	250

Based on extracting 0.5 grams, final volume 0.5 ml and 0.3 ml, injecting 5.0 μ l on the GC.

BEGIN
 LAST LINE
 OF TEXT

BOTTOM OF
 IMAGE AREA
 OUTSIDE
 DIMENSION
 FOR TABLES
 AND ILLUSTRATIONS

TABLE A10. DETECTION LIMITS IN PPB WHOLE BLOOD

	Theoretical	Practical
alpha-BHC	0.5	1.0
beta-BHC	2.5	1.0
Heptachlor	0.5	1.0
p,p'-DDT	2.5	3.0
o,p'-DDT	2.5	3.0
p,p'-DDE	1.2	2.0
o,p'-DDE	2.5	3.0
p,p'-DDD	2.5	3.0
Endrin	2.5	3.0
Thiodan	5.0	8.0
Lindane	0.5	1.0
Dieldrin	1.0	2.0
Heptachlor Epoxide	0.5	1.0
Oxychlordane	0.5	1.0
Mirex	12.5	15.0
Hexachlorobenzene	0.2	1.0
t-Nonachlor	0.5	1.0
Aldrin	0.5	1.0
Chlordane	12.5	15.0
Dacthal	2.5	3.0
Methoxychlor	12.5	15.0
Aroclor 1254	62.5	75.0

Based on extracting 2.0 grams of sample, with a final volume of 1.0 ml and 5.0 μ l injected on the GC.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

TABLE A11. DETECTION LIMITS IN PPB (WET WT.) FOR SLUDGE SAMPLES FROM WASTE-WATER TREATMENT PLANT AND NOT FED TO CATTLE IN FEEDING TRIAL

	Theoretical	Practical
p,p'-DDT	12.5	25.0
o,p'-DDT	12.5	25.0
p,p'-DDE	6.2	12.4
o,p'-DDE	12.5	25.0
p,p'-DDD	12.5	25.0
alpha-BHC	1.2	2.4
beta-BHC	12.5	25.0
Heptachlor	2.5	5.0
Endrin	12.5	25.0
Lindane	2.5	5.0
Dieldrin	5.0	10.0
Heptachlor Epoxide	2.5	5.0
Oxychlordane	2.5	5.0
Mirex	62.5	125.0
HCB	1.2	2.4
t-Nonachlor	2.5	5.0
Aldrin	2.5	5.0
Chlordane	62.5	125.0
Methoxychlor	62.5	125.0
Arochlor 1254	312.0	624.0

Based on extracting 2.0 gm of sample, a final volume of 5.0 ml and "plugging" 5.0 µl on the GC.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA.
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

FROM
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

TABLE A12. DETECTION LIMITS IN PPB (DRY WEIGHT) FOR SOIL SAMPLES

	Theoretical	Practical
p,p'-DDT	12.5	25.0
o,p'-DDT	12.5	25.0
p,p'-DDE	6.2	12.4
o,p'-DDE	12.5	25.0
p,p'-DDD	12.5	25.0
alpha-BHC	1.2	2.4
beta-BHC	12.5	25.0
Heptachlor	2.5	5.0
Endrin	12.5	25.0
Lindane	2.5	5.0
Dieldrin	5.0	10.0
Heptachlor Epoxide	2.5	5.0
Oxychlordane	2.5	5.0
Mirex	62.5	125.0
HCB	1.2	2.4
t-Nonachlor	2.5	5.0
Aldrin	2.5	5.0
Chlordane	62.5	125.0
Methoxychlor	62.5	125.0
PCB	312.0	624.0
Thiodan	2.5	5.0

Based on extracting 2.0 gm of sample, a final volume of 5.0 ml and "plugging" 5.0 μ l on the GC.

FROM
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA;
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

TABLE A13 PERCENT LIPID IN SAMPLES FROM LBR CATTLE (E), CONTROL RANCH CATTLE (C), AND CATTLE FED METRO DENVER SLUDGE

<u>Kidney</u>	<u>Percent Lipid</u>	<u>Liver</u>	<u>Percent Lipid</u>	<u>Fat</u>	<u>Percent Lipid</u>
<u>Lowry Bombing Range Cattle:</u>					
E1	1.9	E1	1.3	E1	62.7
E2	0.6	E2	1.3	E2	51.1
E3	1.0	E3	2.9	E3	58.8
E4	1.4	E4	3.2	E4	67.0
E5	1.3	E5	1.1	E5	75.0
E6	1.6	E6	2.7	E6	28.2
E7	1.4	E7	0.9	E7	48.2
E8	1.2	E8	1.9	E8	48.3
E9	4.3	E9	0.9	E9	44.5
E10	1.8	E10	2.4	E10	49.2
E11	1.2	E11	1.8	E11	38.9
E12	1.0	E12	3.6	E12	52.3
<u>Control Ranch Cattle:</u>					
C1	0.9	C1	2.2	C1	74.1
C2	0.9	C2	0.9	C2	64.3
C3	1.1	C3	3.1	C3	59.4
C4	1.3	C4	10.2	C4	70.5
C5	1.0	C5	2.7	C5	56.4
C6	1.0	C6	2.4	C6	56.2
<u>Fat</u>	<u>Percent Lipid</u>	<u>Muscle</u>	<u>Percent Lipid</u>		
<u>0% Sludge Diet:</u>		<u>0% Sludge Diet:</u>			
F-0-1012	76	M-0-1012	1.0		
F-0-1020	77	M-0-1020	0.9		
F-0-1021	62	M-0-1021	1.9		
F-0-1022	67	M-0-1022	0.2		
F-0-1025	59	M-0-1025	1.9		
F-0-1028	74	M-0-1028	1.7		
<u>12% Sludge Diet:</u>		<u>12% Sludge Diet:</u>			
F-12-1011	68	M-12-1001	0.2		
F-12-1007	69	M-12-1007	0.2		
F-12-1015	62	M-12-1015	0.5		
F-12-1026	79	M-12-1026	0.1		
F-12-1029	79	M-12-1029	0.3		
F-12-1030	71	M-12-1030	0.8		

TABLE A14. PERCENT LIPID IN SAMPLES FROM CATTLE FED METRO DENVER SEWAGE SLUDGE

<u>Kidney</u>	<u>Percent Lipid</u>	<u>Muscle</u>	<u>Percent Lipid</u>
<u>0% Sludge Diet:</u>		<u>0% Sludge Diet:</u>	
K-0-1012	1.5	L-0-1012	0.3
K-0-1020	1.5	L-0-1020	2.2
K-0-1021	1.2	L-0-1021	2.2
K-0-1022	0.3	L-0-1022	1.7
K-0-1025	1.1	L-0-1025	1.5
K-0-1028	1.3	L-0-1028	1.8
<u>12% Sludge Diet:</u>		<u>12% Sludge Diet:</u>	
K-12-1001	1.9	L-12-1001	2.0
K-12-1007	1.2	L-12-1007	2.0
K-12-1015	1.8	L-12-1015	2.7
K-12-1026	1.5	L-12-1026	1.4
K-12-1029	2.0	L-12-1029	1.5
K-12-1030	1.0	L-12-1030	2.0

APPENDIX B

SOIL PROFILE DESCRIPTION AND HEAVY METAL CONCENTRATIONS OF SOILS AND PLANTS AT LOWRY BOMBING RANGE AND CONTROL RANCH

(Color for moist soil unless otherwise stated)

Profile 1. Soil profile described at control ranch.

table
Soil	: Bresser
Area	: Arapahoe County, Colorado
Vegetation	: Pasture, dominantly buffalo grass and blue grama
Parent Material:	Loess
Topography	: About 2% slope facing N.W.
Ap	0-15 cm, very dark grayish brown (10YR3/2) sandy loam; weak fine subangular blocky structure; firm when dry, friable when moist; abundant roots; pH 7.1; abrupt boundary.
A₃	15-23 cm, very dark grayish brown (10YR3/2) sandy loam; weak fine subangular blocky structure; firm when dry, friable when moist; roots common; pH 7.3; clear smooth boundary.
B_{2t}	23-46 cm, dark brown (10YR3/3) sandy clay loam; moderate medium prismatic; very hard when dry, friable when moist; few roots; pH 6.5; clear smooth boundary.
B₃	46-71 cm, dark brown (10YR4/3) sandy loam; moderately weak medium prismatic; hard when dry, friable when moist; few roots; pH 6.7; clear smooth boundary.
C₁	71-117 cm, dark brown (10YR4/3) loamy sand; single grain; friable when moist and dry; few roots; pH 7.0; abrupt smooth boundary.
IIC_{ca}	117-145 cm, reddish brown (2.5YR4/4) sandy loam; massive; friable when moist and dry; carbonate mottles light gray (10YR7/1); pH 7.3; abrupt wavy boundary.

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLU-
STRATIONS

Profile I. Heavy metal concentrations.

Horizon	Zn		Cd		Cu		Ni		Pb	
	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
A _p	14.8	0.28	0.22	0.04	2.50	0.44	2.75	0.42	5.50	0.50
A ₃	16.8	0.06	0.32	0.03	2.50	0.32	10.0	0.50	5.50	0.52
B _{2t}	21.0	0.16	0.22	0.02	2.75	0.32	7.25	0.54	6.75	0.50
B ₃	12.5	0.20	0.18	0.03	2.75	0.44	5.50	0.36	5.00	0.52
C ₁	6.2	0.12	0.20	0.02	1.50	0.20	2.50	0.18	2.25	0.38
II _{Cca}	13.8	0.14	0.35	0.01	2.50	0.24	4.25	0.16	7.50	0.36
Pasture Grass	9.75		0.28		2.50		2.75		9.75	

Profile II. Soil profile described at control ranch.

- Soil : Alluvium
- Area : Arapahoe County, Colorado
- Vegetation : Pasture, western wheat grass and blue grama under cottonwood trees.
- Topography : Flood plain of intermittent stream, 0-1% slope.
- A_p 0-18 cm, very dark gray (10YR3/1) gravelly loamy sand; medium platy structure, platy from deposition; firm when dry, friable when moist; abundant roots; pH 7.0; abrupt smooth boundary.
- IIA_b 8-38 cm, very dark gray (10YR3/1) loam; weak thin moderate platy structure; platy from deposition; very hard when dry; roots common; carbonate mottles; pH 6.8; abrupt smooth boundary.
- C₁ 38-89 cm, very dark grayish brown (10YR3/2) loamy sand; weak medium subangular blocky structure, some platiness from deposition; friable when dry, very friable when moist; faint iron mottles; pH 7.1; few roots; pH 7.0; gradual smooth boundary.
- C₂ 89-142 cm, light brownish gray (10YR6/2) coarse sand; single grain, stratified; pH 7.2; roots few.

Profile II. Heavy metal concentrations.

Horizon	Zn		Cd		Cu		Ni		Pb	
	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
A _p	6.00	0.42	0.12	0.04	1.75	0.30	1.25	0.26	3.50	0.70
II _{Ab}	18.1	0.24	0.24	0.05	3.25	0.74	5.12	0.34	9.75	1.14
C ₁	6.25	0.16	0.05	0.02	1.25	0.22	2.50	0.16	3.00	0.40
C ₂	2.00	0.16	0.05	0.02	1.50	0.16	1.00	0.12	3.25	0.24
Pasture Grass	16.8		0.28		2.75		0.25		2.00	

Profile III. Soil profile described at Lowry Bombing Range in an area where no sludge had been applied.

Area : Arapahoe County, Colorado

Vegetation : Pasture, dominantly buffalo grass and blue grama.

Parent Material: Loess

Topography : 3 to 4% slope facing north.

- A₁ 0-10 cm, very dark grayish brown (10YR3/2) silt loam, weak fine granular structure; slightly hard dry, friable when moist; pH 7.1; abundant roots; clear smooth boundary.
- B_{21t} 10-23 cm, very dark grayish brown (10YR3/2) silty clay loam; moderate medium prismatic breaking to moderate fine subangular blocky; hard when dry, firm when moist; pH 7.1; abundant roots; clear smooth boundary.
- B_{22t} 23-38 cm, dark brown (10YR4/3) silty clay loam; moderate medium prismatic breaking to moderate fine subangular blocky; hard when dry, firm when moist; pH 6.9; abundant roots, clear smooth boundary.
- B_{23tca} 38-63.5 cm, dark yellowish brown (10YR4/4) silt loam; moderate medium prismatic breaking to moderate fine subangular blocky; hard when dry, firm when moist; pH 7.3; carbonate mottles light gray (10YR7/1); abundant roots; gradual smooth boundary.
- B_{24ca} 63.5-114 cm, dark yellowish brown (10YR4/4) clay; strong coarse prismatic structure; very hard when dry, very firm when moist; pH 7.8; carbonate mottles light gray (10YR7/1); few roots; continuous clay skins; clear smooth boundary.
- II_C 114-129.5 cm, olive (10YR4/3) clay; massive structure; very hard when dry, very firm when moist; pH 7.2; carbonate mottles light gray (10YR7/1); few roots.

Profile III. Heavy metal concentrations.

Horizon	mg/kg (dry weight)									
	Zn		Cd		Cu		Ni		Pb	
	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
A ₁	47.0	0.56	0.12	0.09	10.0	1.38	12.5	0.68	23.0	1.56
B _{21t}	49.0	0.22	0.45	0.07	7.00	1.50	12.5	0.78	16.2	1.04
B _{22t}	42.2	0.08	0.42	0.03	7.50	1.26	12.5	0.30	12.5	0.82
B _{23tca}	40.5	0.18	0.25	0.02	9.25	1.26	12.2	0.16	13.0	0.82
B _{24tca}	49.5	0.26	0.12	0.02	18.0	2.32	13.0	0.14	14.0	1.08
II _C	87.5	0.36	0.55	0.07	18.2	1.46	16.7	0.26	14.0	0.98
Pasture Grass	10.5		0.05		1.50		0.25		1.12	

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
PAGE

TOP OF
IMAGE
AREA

Profile IV. Soil profile described at Lowry Bombing Range in an area where no sludge had been applied.

ENCLOSURE
HEAD
SECTION
LINE

Soil : Fondis
Area : Arapahoe County, Colorado
Vegetation : Pasture, dominantly buffalo grass and blue grama.
Parent Material: Loess
Topography : 3 to 4% slope facing west.

A₁ 0-10 cm, dark brown (10YR4/3) silt loam; weak fine granular structure; firm when dry, friable when moist; pH 7.5; abundant roots; clear smooth boundary.

B_{21t} 10-30.5 cm, dark brown (10YR3/3) silty clay loam; strong medium prismatic structure; continuous clay coats; very hard when dry, firm when moist; pH 7.2; abundant roots; clear smooth boundary.

B_{22t} 30.5-41 cm, dark brown (10YR4/3) silty clay loam; moderate medium prismatic breaking to strong medium angular blocky; continuous clay coats; very hard when dry, firm when moist; pH 7.8; abundant roots; clear smooth boundary.

B_{23tca} 41-57 cm, dark yellowish brown (10YR4/4) silty clay loam; strong medium prismatic structure; continuous clay coats; very hard when dry, firm when moist; pH 8.1; carbonate mottles light gray (10YR7/1); roots common; gradual smooth boundary.

B_{24ca} 57-89 cm, dark yellowish brown (2.5YR4/4) silty clay; strong medium prismatic structure; continuous clay coats; very hard when dry, firm when moist; pH 7.8; carbonate mottles light gray (10YR7/1); few roots; gradual smooth boundary.

B_{3ca} 89-119 cm, reddish brown (2.5YR4/4) silty clay; strong medium prismatic structure; clay coats; very hard when dry, firm when moist; pH 7.5; carbonate mottles light gray (10YR7.1); very few roots; clear smooth boundary.

C_{ca} 119-142 cm, olive (5Y4/3) clay; massive; hard when dry, firm when moist; clay coats; pH 7.6; carbonate mottles light gray (10YR7/1); iron oxide mottles strong brown (7.5YR5/6); very few roots.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
INSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

Profile IV. Heavy metal concentrations.

Horizon	mg/kg (dry weight)									
	Zn		Cd		Cu		Ni		Pb	
	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
A ₁	44.5	3.02	0.22	0.14	9.75	2.40	11.0	1.14	19.0	1.66
B _{21t}	49.2	0.16	0.50	0.05	7.25	1.78	20.0	0.72	17.2	0.94
B _{22t}	51.0	0.20	0.38	0.02	8.25	1.56	18.2	0.36	16.8	0.90
B _{23t}	34.8	0.14	0.28	0.03	7.50	1.26	10.0	0.22	12.0	0.72
B _{24t}	33.2	0.10	0.70	0.01	6.00	1.06	7.50	0.22	15.5	0.78
B _{3ca}	30.2	0.18	0.25	0.01	6.50	1.30	6.50	0.16	14.0	0.82
C _{ca}	40.6	0.52	0.25	0.02	8.00	1.50	3.62	0.16	15.2	0.94
Pasture Grass	8.00		0.12		2.75		1.25		3.62	

Profile V. Soil profile described at Lowry Bombing Range in an area where approximately 130 tons/ha of sludge (dry weight) had been applied. Profile V approximately 100 meters from Profile III.

Soil : Fondis
 Area : Arapahoe County, Colorado
 Vegetation : Crop land (winter wheat)
 Parent Material: Loess
 Topography : 3 to 4% slope facing north.

A_p 0-30 cm, dark brown (10YR3/3) silt loam; weak fine granular structure; friable when moist; pH 6.4; few roots; abrupt boundary.

B_{22t} 30-36 cm, dark brown (10YR4/3) silty clay loam; moderate medium prismatic breaking to moderate fine subangular blocky; hard when dry, firm when moist; pH 6.9; few roots; clear smooth boundary.

B_{23tca} 36-51 cm, dark yellowish brown (10YR4/4) silt loam; moderate medium prismatic breaking to moderate fine subangular blocky; very firm when dry, firm when moist; pH 6.9; carbonate mottles light gray (10YR7/1) few roots; gradual smooth boundary.

B_{24tca} 51-107 cm, dark yellowish brown (10YR4/4) clay; strong coarse prismatic structure; very hard when dry, very firm when moist; pH 7.6; carbonate mottles light gray (10YR7/1); few roots; continuous clay skins; clear smooth boundary.

II_c

107-121 cm, olive (5Y4/3) clay; massive structure, very hard when dry, very firm when moist; pH 7.2; carbonate mottles light gray (10YR7/1); few roots.

Profile V. Heavy metal concentrations.

Horizon	mg/kg (dry weight)									
	Zn		Cd		Cu		Ni		Pb	
	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
A	52.5	2.54	0.58	0.17	13.5	2.72	11.5	1.38	18.2	1.42
P										
B _{22t}	69.5	0.32	0.62	0.09	16.5	2.24	21.2	1.78	16.0	1.50
B _{23tca}	46.5	0.14	0.65	0.04	7.50	1.54	7.50	0.60	17.2	1.06
B _{24tca}	55.5	0.22	0.65	0.03	12.2	1.68	16.2	0.66	14.2	1.02
Plant Tissue*	54.0		0.70		12.0		1.50		2.40	

* Winter Wheat - Sampled in the fall.

Profile VI. Soil profile described at Lowry Bombing Range in an area where approximately 71 tons/ha (dry weight) of sludge had been applied. Profile VI approximately 100 meters from Profile IV.

Soil : Fondis

Area : Arapahoe County, Colorado

Vegetation : Field fallow

Parent Material: Loess

Topography : Approximately 3% slope facing southwest.

A_p 0-15 cm, dark brown (10YR3/3) silt loam; weak fine granular; friable when moist; pH 7.0; few roots; abrupt boundary.

B_{22t} 15-30 cm, dark brown (10YR4/3) silty clay loam; moderate medium prismatic breaking to strong medium subangular blocky; continuous clay coats; very hard when dry, firm when moist; pH 7.2; few roots; clear smooth boundary.

B_{23tca} 30-48 cm, dark yellowish brown (10YR4/4) silty clay loam; strong medium prismatic structure; continuous clay coats; very hard when dry, firm when moist; pH 7.5 carbonate mottles light gray (10YR7/1); few roots; gradual smooth boundary.

B24tca

48-66 cm, dark yellowish brown (10YR4/4) silty clay; strong medium prismatic; continuous clay coats; very hard when dry, firm when moist; pH 7.5; carbonate mottles light gray (10YR7/1); few roots; gradual smooth boundary.

B3ca

66-91 cm, reddish brown (2.5YR4/4) silty clay; strong medium prismatic structure; clay coats; very hard when dry, firm when moist; pH 7.9; carbonate mottles strong brown (7.5YR5/6); very few roots.

Profile VI. Heavy metal concentrations.

Horizon	mg/kg (dry weight)									
	Zn		Cd		Cu		Ni		Pb	
	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
A _p	83.0	19.1	1.36	0.66	23.4	13.8	20.5	5.43	31.7	5.25
B _{22t}	61.5	0.58	0.60	0.08	11.2	2.24	19.0	1.62	16.0	1.74
B _{23tca}	48.0	0.20	0.65	0.04	11.2	1.58	15.0	0.62	14.0	1.14
B _{24tca}	40.0	0.16	0.55	0.01	10.0	1.28	13.5	0.42	11.75	0.96
B _{3ca}	29.2	0.18	0.85	0.004	6.00	0.80	7.50	0.50	7.00	0.42
C _{ca}	21.2	0.18	1.12	0.04	4.75	0.48	6.00	0.40	7.50	0.34

Profile VII. Soil profile described at Lowry Bombing Range in an area where no sludge had been applied.

Soil : Renohill

Area : Arapahoe County, Colorado

Vegetation : Pasture, dominantly buffalo gras and blue grama.

Parent Material: Loess

Topography : About 6% slope facing east.

A_p 0-15 cm, dark brown (10YR3/3) loam; moderate fine granular; slightly hard when dry, friable when moist; pH 7.9; abundant roots; abrupt boundary.

B_{21t} 15-53 cm, dark yellowish brown (10YR4/4) loam; weak medium prismatic; slightly hard when dry, friable when moist; pH 7.4; few carbonate specks; abundant roots; clear smooth boundary.

B_{22tca} 53-69 cm, dark brown (10YR4/3) silty clay loam; moderate medium to coarse prismatic; hard when dry, firm when moist; carbonate specks; pH 7.8; roots common; clear smooth boundary.

B₃

69-76 cm, reddish brown (2.5YR4/4) loam; strong medium angular blocky; very hard when dry; firm when moist; pH 6.7; carbonate mottles light gray (10YR7/1); roots common.

Profile VII. Heavy metal concentrations.

Horizon	mg/kg (dry weight)									
	Zn		Cd		Cu		Ni		Pb	
	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
A _p	45.5	0.58	0.78	0.07	13.2	0.68	9.75	0.26	17.5	2.34
B _{21t}	50.2	0.14	0.75	0.02	12.8	0.52	9.25	0.16	16.8	0.58
B _{22tca}	51.2	0.18	0.56	0.01	13.4	0.64	9.75	0.22	15.0	0.65
B _{3ca}	73.5	0.26	0.65	0.02	23.8	0.68	8.75	0.12	17.8	0.82
Plant Tissue	7.00		0.22		2.81		0.56		3.25	

Profile VIII. Soil profile described at Lowry Bombing Range in an area where approximately 295 tons/ha (dry weight) of sludge had been applied. Profile approximately 100 meters from Profile VII.

Soil : Renohill
 Area : Arapahoe County, Colorado
 Vegetation : Field fallow
 Parent Material: Loess
 Topography : About 4% slope facing east.

A_p 0-23 cm, dark brown (10YR3/3) loam; moderate fine granular; slightly hard when dry, very friable when moist; pH 6.5; abundant roots; clear smooth boundary.

B_{21t} 23-46 cm, dark yellowish brown (10YR4/4) loam; weak medium prismatic; slightly hard when dry, friable when moist; pH 6.8; few carbonate specks; abundant roots; clear smooth boundary.

B_{22tca} 46-69 cm, dark brown (10YR4/3) silty clay loam; moderate medium to coarse prismatic; hard when dry, firm when moist; carbonate specks; pH 7.1; roots common, clear smooth boundary.

B_{3ca} 69-91 cm, reddish brown (2.5YR4/4) loam; strong medium angular blocky; very hard when dry, firm when moist; pH 7.8; carbonate mottles light gray (10YR7/1); roots common; gradual smooth boundary.

C₁ca

91-114 cm, reddish brown (2.5R4/4) loam; moderate coarse platy; very hard when dry, firm when moist; pH 7.8; carbonate mottles yellowish brown (10YR5.6); few roots; gradual smooth boundary.

C₂ca

114-147 cm, reddish brown (2.5YR4/4) loam to clay loam; moderate coarse platy; saprolitic; very hard when dry, friable when moist; pH 7.6; carbonate mottles yellowish brown (2.5YR4/4); very few roots.

Profile VIII. Heavy metal concentrations

Horizon	mg/kg (dry weight)									
	Zn		Cd		Cu		Ni		Pb	
	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
A _p	90.9	11.1	1.35	0.52	27.1	7.58	15.0	1.88	31.8	3.80
B ₂₁ tca	53.8	0.26	0.22	0.02	14.5	0.82	7.50	0.20	11.5	0.54
B ₂₂ tca	61.0	0.30	0.28	0.03	13.2	1.00	8.50	0.18	13.0	0.88
B ₃ ca	70.5	0.50	0.72	0.03	11.2	0.86	5.75	0.16	19.5	1.10
C ₁ ca	74.0	0.62	0.42	0.03	11.8	0.54	5.75	0.12	15.5	1.10
C ₂ ca	56.0	0.50	0.18	0.05	17.0	0.76	4.25	0.10	12.0	1.02

Profile IX. Soil profile described at the Lowry Bombing Range in an area where more than 448 tons/ha (dry weight) had been applied.

Soil : Alluvial

Area : Arapahoe County, Colorado

Vegetation : Crop land (winter wheat)

Parent Material: Alluvium

Topography : 1% slope, flood plain of intermittent stream.

A_p 0-23 cm, very dark grayish brown (10YR3/3) silt loam; moderate medium granular; very friable; pH 7.1; roots common; abrupt boundary.

B₂₂t 23-76 cm, dark grayish brown (10YR3/2) clay; moderate medium subangular blocky; firm; pH 7.1; roots common; smooth clear boundary.

B₂₃tca 76-94 cm, dark grayish brown (10YR4/2) clay; moderate medium subangular blocky; firm when dry, friable when moist; pH 7.0; abundant carbonate mottles light gray (10YR7/1); few roots; smooth gradual boundary.

B24t

94-102 cm, weak red (2.5YR4/2) clay; moderate medium subangular blocky; hard when dry, friable when moist; pH 7.6; abundant carbonate mottles light gray (10YR7/1); few roots; abrupt boundary with much tonguing.

II_{c1}

102-110 cm, reddish brown (2.5YR4/4) fine sand; massive; friable; pH 7.4.

Profile IX Heavy metal concentrations.

Horizon	mg/kg (dry weight)									
	Zn		Cd		Cu		Ni		Pb	
	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA	Total	DTPA
Ap	103.2	15.3	1.52	0.52	35.0	11.5	20.0	2.70	46.0	6.62
B22t	56.2	0.30	0.45	0.05	13.0	2.04	10.8	0.62	19.8	2.60
B23tca	50.8	0.28	0.50	0.04	13.0	1.92	11.8	0.64	19.2	2.12
B24tca	28.5	0.34	0.32	0.03	5.50	1.82	6.75	0.28	15.0	1.64
II _{c1}	27.5	0.38	0.35	0.02	5.00	1.96	7.50	0.24	16.2	1.76
Plant *										
Tissue	102.		2.45		12.5		4.20		<0.65	

* Winter wheat sampled in the fall.

APPENDIX C

NECROPSY REPORT OF STEERS FED 0% AND 12%
FT. COLLINS SEWAGE SLUDGE FOR 3-MONTHSControl Group

- 77D6220 - #1087 - No lesions were seen.
- 77D1292 - #1272 - A 3-4 cm abscess involved a retropharyngeal lymph node. An abscess was found in the right diaphragmatic lobe of the lung. Lung parenchyma contained numerous 2-3 mm raised red foci (hemorrhages).
- 77D6222 - #1171 - Mesenteric and retropharyngeal lymph nodes were reddened. Numerous small abscesses were found in the liver. These were encapsulated and contained tenacious yellow-white material.
- 77D6223 - #1204 - A large, encapsulated abscess containing creamy, white pus was present on the surface of the liver.
- 77D6224 - # 970 - No lesions were seen.
- 77D6225 - #1205 - Numerous depressed white, firm scarred areas were seen in the liver, but no exudate was present in these foci (old healed abscess).

Sludge Exposed Group

- 77D6226 - #1058 - Numerous 2-3 mm firm, white, raised nodules were seen in the mucosa of the esophagus. Mesenteric lymph nodes were slightly enlarged and reddened. Numerous petechia were seen in the lungs.
- 77D6227 - #1170 - Mesenteric lymph nodes were slightly enlarged and petechia were seen in the lungs.
- 77D6228 - #1257 - Fibrous adhesions of the reticulum to the diaphragm were found. A wire was found penetrating the wall of the reticulum in this area. Several 2-3 cm abscesses filled with yellowish caseous material were found in the liver

77D6231 - #1506 - No lesions were seen.

All the animals in the treatment and control groups were evaluated at an antemortem inspection immediately prior to slaughter. Evaluations for the antemortem inspection were based on the following general criteria:

Waste Elimination (volume,
consistency, composition)
Skin Color
Visible Mucous Membrane
Locomotion, Stance
Abnormal Gait or Lameness
Abnormal Articular Sounds

Tissues and organs collected from the animals at necropsy included: cortical bone (radius), articular surface (carpus), liver, rumen, reticulum, omasum, abomasum, small intestine, large intestine, kidney, lung, spleen, abdominal fat, subcutaneous fat, skin (brisket region) and other tissues or organs appearing abnormal at necropsy.

ANTEMORTEM INSPECTION

Antemortem observations included the absence of switches and distal portions of the tail. The haircoat was shorter but uniform on the right flank, operative site for hepatic biopsies taken during the test period. The cattle were uniform within each of the four treatment groups with one exception, cow Number 322. This cow was in the sludge withdrawal treatment group and had a slightly roughened haircoat, and was approximately 200 pounds lighter than the other three cows in this group.

NECROPSY

Minimal gross observations were noted at necropsy of the steers and cows killed after being fed a diet containing 12.5% sewage sludge for 279 days. Two steers and three cows had stellate areas of capsular fibrosis at the hepatic biopsy site varying in diameter from 3-8 cm and loosely attached by fibrin strands to adjacent peritoneum.

Steers and cows fed control ration for 279 days before slaughter displayed areas of capsular fibrosis and scarring at the hepatic biopsy site in four steers and similar scarring in three cows. No other lesions were noted at necropsy which included examination of organs in the thoracic and peritoneal cavities, the oral cavity, the articular surfaces, the joint spaces, the skeletal musculature, and the dental arcades.

Necropsies of steers and cows fed a diet containing 12.5% sewage sludge for 279 days and control ration for 120 days revealed areas of capsular fibrosis at the hepatic biopsy site in three steers and three cows. In two steers these areas extended into the hepatic parenchyma 4-5 cm and there were small adhesions to the adjacent peritoneum. One cow (Number 322) had hepatic capsular fibrosis with adhesions to the diaphragm and adjacent peritoneum. Number 322 also had a firm multinodular encapsulated abdominal mass in the peripancreatic mesentery that measured 25 cm in its greatest dimension. The cut surface of this mass contained zones of necrosis, fibrous connective tissue and fat. In comparison to the other sludge withdrawn cows, Number 322 had a reduced amount of subcutaneous and abdominal fat.

Two steers and two cows on control rations for 399 days had scars in the liver due to hepatic biopsies taken during the test. The gross observations of these areas included small adhesions to the adjacent peritoneal surfaces.

HISTOPATHOLOGIC OBSERVATIONS

Histopathologic observations are presented in Tables C1 and C2. A neurofibroma in the abdominal cavity of Cow Number 322 (279 days 12.5% sewage sludge, 120 days control ration) was diagnosed. This tumor was rich in connective tissue components and appeared to originate from perineural

connective tissue cells. The tumor contained spindle-shaped cells arranged in herringbone, palisading and whorling patterns, separated by connective tissue septae.

Pericholangitis (mild inflammation surrounding the portal triads of the liver) occurred in very slight to slight severity in at least one animal of each treatment group. An occasional animal had evidence of a mononuclear cell infiltrate in the interstitial of the kidneys. The hepatic biopsy site contained scars with fibrous connective tissue, acute and chronic inflammatory cells, small foci of necrosis and hemosiderin laden macrophages.

DISCUSSION

There was no evidence at antemortem inspection, necropsy or at histopathologic examination of a treatment related effect in cattle fed the diet containing 12.5% sewage sludge for 279 days or 279 days followed by 120 days of control ration (no sludge). The observations made at antemortem inspection, necropsy and histopathologic examination of selected tissues were related to the experimental procedure, i.e. the distal portion of the tail was amputated during the study to collect bone samples, the minimal pericholangitis and mononuclear cell infiltrate of the kidneys is a common finding in animals of similar age and medical history. The hepatic scarring at the biopsy sites is an expected sequelae to a biopsy of this organ.

The neurofibroma that occurred in the peripancreatic mesentery of Cow Number 322 was a tumor of peripheral nerves that is not uncommon in cows of similar age and background.

TABLE C1. HISTOPATHOLOGIC OBSERVATIONS MADE ON SELECTED TISSUES FROM CATTLE FED A DIET CONTAINING 12.5% SEWAGE SLUDGE FOR 279 DAYS, OR THE CONTROL RATION FOR 279 DAYS

	Control Steers						Control Cows						Treated Steers						Treated Cows					
	313	315	316	326	314	317	324	325	319	320	322	327	312	318	321	323	312	318	321	323	312	318	321	323
Accession Number 80-	34	61	74	6422	53	107	4317	5222	475	505	555	B-70	2	329	5239	4076	2	329	5239	4076	2	329	5239	4076
Animal Number	STR	STR	STR	STR	COW	COW	COW	COW	STR	STR	STR	STR	COW	COW	COW	COW	STR	STR	STR	STR	COW	COW	COW	COW
Sex	STR	STR	STR	STR	COW	COW	COW	COW	STR	STR	STR	STR	COW	COW	COW	COW	STR	STR	STR	STR	COW	COW	COW	COW
Musculoskeletal Sys.																								
Cortical Bone	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Articular Surface	NR	NR	TNP	TNP	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	TNP	NR	TNP	NR
Gastrointestinal Sys.																								
Liver	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pericholangitis																								
Rumen	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Reticulum	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Abomasum	NR	NR	TNP	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Small Intestine	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Large Intestine	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Urinary Sys.																								
Kidney	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mineralization, multifocal																								
Mononuclear cell, infiltrate																								
Respiratory Sys.																								
Lung	TNP	TNP	TNP	TNP	TNP	TNP	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	TNP	TNP	NR	NR
Hemopoietic Sys.																								
Spleen	NR	NR	NR	NR	NR	NR	TNP	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	TNP	TNP	TNP	NR
Other																								
Abdominal Fat	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP	TNP
Hepatatic Biopsy Site																								
Inflammation, chronic	3	3	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

KEY: NR = Tissue Present, Not Remarkable
 TNP = Tissue Not Present
 Severity
 1 = Very Slight
 2 = Slight or Small
 3 = Moderate
 4 = Severe

TABLE C2. HISTOPATHOLOGIC OBSERVATIONS MADE ON SELECTED TISSUES FROM CATTLE FED A DIET CONTAINING 12.5% SEWAGE SLUDGE FOR 279 DAYS FOLLOWED BY 120 DAYS ON CONTROL RATION, OR THE CONTROL RATION FOR 399 DAYS

	Treated Steers						Treated Cows						Control Steers						Control Cows					
	005	006	007	008	001	002	003	004	013	014	015	016	009	010	011	012	009	010	011	012	009	010	011	012
Accession Number 80-	456	664	44	20	4147	322	59	101	66	46ST	7541	49	139	4287	46	298	139	4287	46	298	139	4287	46	298
Animal Number	STR	STR	STR	STR	COW	COW	COW	COW	STR	STR	STR	STR	COW	COW	COW	COW	STR	STR	STR	STR	COW	COW	COW	COW
Sex																								
Musculoskeletal Sys.																								
Cortical Bone	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Articular Surface	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Gastrointestinal Sys.																								
Liver	1	NR	1	1	2	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Pericholangitis																								
Rumen	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Reticulum	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Abomasum	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Omasum	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Small Intestine	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Large Intestine	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Urinary Sys.																								
Kidney	1	2	2	2	NR	NR	NR	2	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Congested																								
Mononuclear cell infiltrate																								
bilateral																								
Respiratory Sys.																								
Lung	2			2																				
Hemopoietic Sys.																								
Spleen	NR	NR	TNP	NR	TNP				NR	NR	NR	NR	TNP	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Congested																								
Integumentary Sys.																								
Skin	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

TABLE C2. (Continued)

Accession Number 80- Animal Number Sex	Treated Steers				Treated Cows				Control Steers				Control Cows			
	005	006	007	008	001	002	003	004	013	014	015	016	009	010	011	012
	456	664	44	20	4147	322	59	101	66	46ST	7541	49	139	4287	46	298
	STR	STR	STR	STR	COW	COW	COW	COW	STR	STR	STR	STR	COW	COW	COW	COW
Other																
Abdominal Fat	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Subcutaneous Fat	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hepatic Biopsy Site																
Inflammation, Chronic		3	4	4	3	4		2								
Modular Mass,																
Peripancreatic Area																
Neurofibroma																
Pancreas																

KEY: NR = Tissue Present, Not Remarkable
TNP = Tissue Not Present

Severity
1 = Very Slight
2 = Slight or Small
3 = Moderate
4 = Severe